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Manual of Analytical Quality Control for Pesticides and Related Compounds in Human and Environmental Samples

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MANUAL OF ANALYTICAL QUALITY CONTROL FOR PESTICIDES AND RELATED COMPOUNDS

IN HUMAN AND ENVIRONMENTAL SAMPLES

A Compendium of Systematic Procedures Designed
To Assist in the Prevention and Control of
Analytical Problems

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FOREWORD

The many benefits of our modern developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This manual provides the pesticide chemist with a systematic protocol for the quality control of analytical procedures and the problems that arise in the analysis of human or environmental media.

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ABSTRACT

This manual provides the pesticide chemist with a systematic protocol for the quality control of analytical procedures and the problems that arise in the analysis of human or environmental media. It also serves as a guide to the latest and most reliable methodology available for the analysis of pesticide residues in these and other sample matrices. The sections dealing with inter- and intra-laboratory quality control, the evaluation and standardization of materials used, and the operation of the gas chromatograph are intended to highlight and provide advice in dealing with many problems which constantly plague the pesticide analytical chemist. Many aspects of the problem areas involved in extraction and isolation techniques for pesticides in various types of samples are discussed. Techniques for confirming the presence or absence of pesticides in sample materials are treated at some length. This highly important area provides validation of data obtained by the more routine analytical procedures. The gas chromatograph, being the principal instrument currently used in pesticide analysis, often requires simple servicing or troubleshooting. A section addressing some of these problems is included. Last, but by no means least in importance, is a short dissertation of the value and need for systematic training programs for pesticide chemists.

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Section 1

GENERAL DESCRIPTION OF PESTICIDE RESIDUE ANALYTICAL METHODS

A pesticide residue analysis usually consists of five steps:

(1) Sampling.

(2) Extraction of the residue from the sample matrix.

(3) Removal of interfering co-extractives ("cleanup").

(4) Identification and estimation of the quantity of residues in the cleaned-up extract, usually at very low levels (e.g., 10^{-9} to 10^{-12} g for gas chromatography). To obtain this sensitivity, selective determinative methods such as chromatography are usually required.

(5) Confirmation of the presence and identity of the residues.

The exact nature of each of these stages is dictated by the specific pesticide(s) and sample substrate involved. A brief discussion of general aspects of these steps follows:

1A SAMPLING

The aim of sampling is to provide a reproduction of a portion of the environment, on a scale that enables the sample to be handled in the laboratory. Analytical results are meaningful only if collected samples are truly representative and meet the goals of the monitoring study or program. The sites, techniques, and frequency of sampling and the size and number of samples must allow the analytical results to be statistically evaluated and replicated at a later time for confirmation. If storage of samples before analysis is necessary, it must be proven that alteration in the nature or amount of pesticide residues does not occur. Samples may be composited or subsampled prior to analysis. The steps in the analytical procedure are influenced significantly by the manner in which the sample is collected, preserved, stored, shipped, and otherwise processed prior to extraction.

1B EXTRACTION PROCEDURES

Environmental and biological samples generally cannot be analyzed directly for pesticide residues because the level of the desired residue is too low, and the levels of interfering constituents are too high. In virtually every modern method of pesticide residue analysis, the compounds of interest are separated from the bulk of the sample matrix by some form of extraction. In most cases, extraction is followed by a cleanup procedure to eliminate, or at least minimize, interfering substances. In both extraction and purification procedures, the fractional recoveries of the compounds must be known, and it must be possible to relate the amounts found in the subsequent assay to the concentrations originally in the sample matrix.

A solvent or mixture of solvents should be used for extraction that is at least 80% efficient, selective enough to require a minimum of cleanup, and does not interfere with the final determination. Simple washing of the whole sample may be adequate for surface residues of foliage or vegetables and fruits, but Soxhlet extractors, blenders, and tumbling or shaking devices are used for most samples. Hexane or hexane-acetone mixtures are typical solvents for nonpolar, fat-soluble organochlorine pesticides; and benzene, chloroform, dichloromethane, or acetonitrile are commonly used for the more polar compounds such as organophosphates and carbamates. Acetonitrile is an excellent general solvent for preliminary extraction of unknown residues of a wide polarity range. The more polar solvents, however, remove greater amounts of co-extractives and may complicate subsequent cleanup steps. Sodium sulfate is sometimes added to help extract the more water-soluble compounds. Exhaustive Soxhlet extraction with an appropriate solvent or mixture of solvents is the most efficient method for many pesticides and sample types and can be used to compare with other proposed procedures.

A given extraction procedure should be validated for each type of sample matrix and for each class of compounds to which it is applied. An extraction procedure suitable for one class of compounds in a given sample may not be suitable for the extraction of a different but closely related class of compounds from the same sample. The nature of the sample matrix influences the effectiveness of the extraction procedure through the toughness, water content, and lipid content. The toughness determines the ease of finely dividing the sample, the water content affects the solubility of pesticides in the extraction solvent, and the lipid content of the sample influences both the amount of solvent and the proportion of nonpolar component required. It is usually desirable to quantitatively extract lipids with the pollutants from environmental samples for ease in reporting analytical results. Optimum extraction conditions in terms of solvent polarity and in the time and manner of contact between sample and extraction solvent should, therefore, be found by recovery studies for each analysis at several concentration levels. Recovery from spiked or fortified samples may not provide valid information about the recovery of endogenous material. Extraction efficiency can be checked most accurately if the laboratory is equipped to biologically incorporate radioactive-labeled parent compounds and/or metabolites in the sample substrate. When polar compounds are involved, hydrolysis to free conjugated residues must be considered before extraction. However, the conditions must be such that the compounds of interest survive the treatment.

1C CLEANUP PROCEDURES

The amount of extract purification (cleanup) required prior to the final determination depends on the selectivity of both the extraction procedure and the determinative method. It is an unusual situation, e.g., with some water samples, when extracts can be directly determined without further treatment. Injection of uncleaned samples into a gas chromatograph can cause extraneous peaks, damage to the peak resolution and efficiency of the column, and loss of detector sensitivity. Impure samples spotted for thin layer chromatography may result in streaked zones or decreased sensitivity of visualizing reagents, while those injected into a liquid chromatograph can greatly shorten the lifetime of an expensive prepacked column. Extracts containing fatty material are especially troublesome. Depending on the extent and nature of the co-extractives and the pesticide residue, partition between immiscible solvents; adsorption chromatography (column or TLC); gel permeation chromatography; chemical destruction of interfering substances with acid, alkali, or oxidizing agents; distillation; sweep co-distillation; and selective photodegradation are most often used for cleanup, either individually or in various combinations.

Plant or crop material is usually extracted with a water-miscible solvent such as acetone or acetonitrile. After dilution with water, the residues are generally partitioned into a solvent such as methylene chloride that can be readily evaporated to dryness. Polar pesticides are only poorly recovered from a surplus of water in this way, and the trend is to keep the residues in organic solution and remove the water co-extracted from the sample. Evaporation of the organic solvent yields good recoveries of even highly polar compounds, which can be analyzed directly by GC with a selective detector or cleaned up further by a multiresidue method such as liquid adsorption chromatography, gel permeation, or sweep co-distillation. For purification of fat extracts, samples are usually partitioned between hexane and acetonitrile, dimethyl formamide, or dimethyl sulfoxide. The latter solvent is used in the widely applicable Wood procedure to elute chlorinated pesticides from a column prepared from a fatty sample mixed with Celite. The lipid content of a particular sample has a significant effect on the recovery of pesticides in solvent partitioning procedures. For example, DDT is recovered more efficiently by acetonitrile partitioning from pure hexane than from a hexane solution containing dissolved fat. This factor can contribute to the variability of recovery in cleanup procedures involving solvent partitioning.

For adsorption chromatography, direct extracts or extracts purified by partitioning are concentrated to a small volume, applied to the top of a Florisil, charcoal, alumina, silica gel, or mixed-adsorbent column, and the pesticides are eluted in fractions by passage of one or more solvents while the co-extractives remain on the column or are eluted in different fractions. Elution of a residue in a certain fraction (selective adsorption) is useful evidence for confirmation of identity. The capacity of a column for co-extractives and the uniformity of activity (elution pattern) from batch to batch are important characteristics of adsorbents used for cleanup. Methods are available for activation and deactivation of different adsorbents and for checking the activity level achieved.

Florisil is still the most widely used adsorbent, and it is involved in cleanup schemes for many fatty and nonfatty samples. However, Florisil is not available in all countries in sufficiently constant quality, and many analysts are becoming increasingly aware that silica gel and alumina are less expensive, are at least as easy to standardize, and provide similarly good results. Another trend is miniaturization of adsorbent cleanup columns. These micro columns, containing, e.g., Florisil or 30% water-deactivated silica gel, are very promising for routine analyses because they offer economy in solvents and materials and reduce health and fire hazards. Sensitivity obtainable is, of course, lower compared to corresponding large sample cleanup procedures.

Chemical destruction methods are extremely useful, but caution must be used in applying them to compounds other than those for which they have specifically been validated. For example, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is commonly determined following alkaline hydrolysis of tissue or extracted lipids, but this treatment completely destroys octachlorodibenzo-*p*-dioxin.

Of the procedures listed above, liquid-liquid partition followed by adsorption chromatography is most often applied for organochlorine pesticides and related compounds (e.g., PCBs) before GC with the relatively unspecific electron capture detector. An automated instrument based on gel permeation chromatography has been shown to efficiently separate chlorinated pesticides and PCBs from the bulk of the lipids extracted from fatty samples, and to be advantageous in terms of convenience and speed of processing large numbers of samples. When specific GC detectors are employed, cleanup of extracts becomes less important. Thus, a suitable extraction procedure combined with a partitioning step is often sufficient for determining organophosphorus and organonitrogen compounds in many samples. HPLC and TLC generally require more effective cleanup steps.

Cleanup procedures should be chosen in terms of practicality, cost, time, and reagent and equipment availability. The methods chosen should be tested to be sure they allow detection and determination of the pesticides of interest at the desired sensitivity level, with recovery of preferably 80-85% or better, and with removal or separation of adequate levels of background interferences. Procedures giving the highest mean recovery of a residue may not necessarily be the best to use if there is a high variation in recovery from sample to sample. A cleanup method giving a moderate but highly reproducible recovery may be a better choice than one giving a high but variable recovery. The nature of the lipids in a sample extract can be important in determining the choice of an adsorbent for liquid column chromatographic cleanup. For example, acidic alumina has a greater capacity for lipids than does basic or neutral alumina.

Concentration of solutions is often required prior to, during, and after cleanup procedures. Great care is necessary when evaporating to low volumes to avoid losses of the pesticide residue, and evaporation to complete dryness is usually inadvisable. Kuderna-Danish evaporators and micro Snyder columns, special block heaters, and rotary-vacuum evaporators are recommended for concentrating solutions containing pesticide residues, and keeper solutions may be added to retard the loss of volatile compounds. All steps in the analytical procedure should be checked for residue loss due to volatilization or degradation by carrying out recovery studies on a spiked control (uncontaminated) sample at different fortification levels. Since concentration factors are often 1000:1 or more, the possibility of interference from the solvent itself must be considered.

Most of the extraction and cleanup procedures available today will yield reliable and reproducible results when practiced by a trained and competent analyst. An important precondition is that a laboratory gain abundant experience with any method that is to be used. The most important analytical methods are those allowing the determination of multiresidues of pesticides and related compounds. Most methods available today, however, are of value only for the parent pesticidal compounds and do not include their significant metabolites. A primary target for future research is the inclusion of the metabolites of toxicological importance in the existing and new multiresidue schemes. In addition, many of the existing multiresidue schemes do not include the many new pesticides, mainly water-soluble and systemic insecticides and fungicides, that have been introduced in the past few years. There is little doubt that a number of analyses for regulatory purposes are routinely performed today for pesticides that have been superseded by other compounds which are not detectable by the procedures in current usage.

1D FINAL DETERMINATION METHODS

Chromatographic methods are by far the most widely used for determination of pesticide residues, followed by spectrophotometric and biological methods. The latter include bioassay and enzymatic techniques that are simple, since they require no cleanup, but are non-specific. Enzyme inhibition, when used as a detection procedure after thin layer chromatography is a sensitive (low ng detection limits) and selective method for certain organophosphorus and carbamate pesticides.

Spectrophotometric methods are generally less sensitive and less selective than gas or thin layer chromatography and are useful mainly as ancillary techniques to gas chromatography for confirmation of residue identity or for quantitation of individual pesticides. If selectivity and sensitivity are adequate, colorimetric methods can advantageously be adapted to automated processes. Fluorescent pesticides and metabolites may be determined by fluorometry, which is more sensitive than visible, UV, or IR methods. Since relatively few pesticides are naturally fluorescent, fluorometry is selective; however, removal of fluorescent impurities is often necessary, and this can be difficult.

Paper chromatography provided the analyst in the late 1950's with the first multiresidue method for separation and identification of pesticides. It has been largely superseded by gas chromatography as the primary determinative procedure and thin layer chromatography (TLC) for screening, semiquantitation, and confirmation. Compared to paper chromatography, TLC offers generally increased resolution, shorter development times, and increased sensitivity. Most pesticide analyses have been performed on 0.25 mm layers of alumina or silica gel, but polyamide and cellulose are also used. Organochlorine compounds are detected at 5-500 ng levels by spraying with ethanolic $AgNO_3$ or incorporation of $AgNO_3$ into the layer followed by irradiation with ultraviolet light. Many organophosphorus and carbamate pesticides are detectable at low ng levels by enzyme inhibition techniques or at higher levels by numerous chromogenic reagents. Fungicides are detectable by bioautography. Polar herbicides and heat-labile, poorly detectable carbamates, which require formation of derivatives prior to gas chromatography, are particularly amenable to analysis by TLC.

Gas chromatography of pesticides is normally carried out on 90 to 200 cm glass columns packed with single and mixed organosilicone and polyester stationary phases ranging from low to high polarity. Among the most used phases are SE-30, QF-1, DC-200, OV-210, OV-17, DEGS, Carbowax 20M, and OV-17/OV-210, SE-30/OV-210, and DC-200/QF-1 mixtures. The chemically stable, low bleed OV series of phases have become quite popular. Samples should be examined on two or three columns of markedly different polarity before results are considered conclusive. A useful series of columns with increasing polarity from which to select an optimum separation is: OV-101 (methyl silicone); OV-17 (methyl/phenyl silicone); OV-210 (methyl/trifluoropropyl silicone); OV-225 (cyanopropyl/phenyl/methyl silicone); and Carbowax 20M (polyethylene glycol).

Glass columns are preferred because they minimize decomposition and are easier to pack for optimum efficiency. After being packed, columns are conditioned at an elevated temperature to minimize liquid phase bleeding and to obtain reproducible chromatograms. In some cases, large quantities of the pesticides being determined are injected initially to improve the response of these compounds. The formation of derivatives in GC residue analysis is a necessity when the analyte is labile or otherwise troublesome or is poorly detected by selective detectors.

Columns loaded with relatively low percentages of liquid phase generally give superior resolution and sensitivity but become contaminated more easily and are more prone to interactions between solutes and the solid support than more heavily coated columns. Certain pesticides, such as DDT and endrin, are subject to degradation in columns under certain conditions, and these conditions should be avoided. Highly inert columns have been prepared by chemically bonding Carbowax 20M to a GC support. These packings are used for GC directly, or after further coating with a liquid phase. Glass capillary columns, with their outstanding separation ability for difficult samples, are being reported much more frequently in residue analysis, even though they are not as tolerant of the injection of "dirty" extracts.

Organochlorine pesticides are usually analyzed with a tritium or ^{63}Ni electron capture detector with DC or pulsed applied voltages. Though this detector is less specific than the other common pesticide detectors, it can detect as low as 10^{-13} g amounts of many halogenated compounds. ^{63}Ni detectors are operable at high temperatures (over 300°C), thus reducing possible problems from contaminants condensing in the detector. Tritium detectors are less expensive, and contaminated foils can be easily changed or removed for cleaning. Commercial devices are available for linearizing EC response over a 10^3-10^5 range of concentration, and the pulsed wide-range ^{63}Ni detector has become especially popular because it can be used with automatic injection systems. Organophosphorus pesticides are detected selectively at ca. 10^{-10}-10^{-11} g levels by the flame photometric detector (FPD) in the phosphorus mode (526 nm), and the FPD has overtaken the thermionic detector as the primary detector for the determination of these compounds. Sulfur-containing pesticides may be selectively detected by the FPD (394 nm) with about one order of magnitude less sensitivity, or at the low ng level with the S-mode of the Hall electrolytic conductivity detector. Nitrogen-containing pesticides are detected selectively with the N-mode of the Hall detector (ca. 10^{-9} g sensitivity) or with the N/P mode of the flameless N-P thermionic detector (ca. 10^{-12} g sensitivity). The N-P thermionic detector also has a mode of operation that is selective for pg levels of only phosphorus-containing compounds, and the Hall detector can be operated selectively for organochlorine compounds at low ng levels. Labile, polar carbamate pesticides or their hydrolysis products are often derivatized with a halogen-containing reagent and the resulting derivative can be sensitively detected with the electron capture detector. Selective detectors have the advantages of simplifying cleanup procedures and aiding residue identification. The mass spectrometer is a unique GC detector in that it is capable of almost specific detection and identification of pesticide residues. It is, however, expensive for routine work.

Samples and standards must be injected into the gas chromatograph using a consistent and reproducible technique. It is advisable that injected volumes of standards and samples be nearly equal and represent 20-80% of the total volume of the syringe used. Syringes must be well cleaned between injections, and injection port septa and liners must be changed regularly. Standards should be injected before and periodically during the analysis of a series of samples. Cleaner samples require fewer standard injections. A pesticide mixture that indicates the overall performance of the GC system should be injected at least once daily.

Modern high performance liquid chromatography (HPLC) is being used increasingly for the final, room temperature determination of polar, involatile, or heat-labile pesticide residues without derivative formation. Analytical columns are 10-50 cm in length and 2-6 mm in internal diameter. For pesticides, they are commonly packed with 5-10 μm particles of a totally porous adsorbent silica gel to which a C_{18} hydrocarbon phase has been chemically bonded (reversed phase chromatography). The mobile phase is pumped through the column at flow rates of 1-2 ml/minute (100-200 atm. pressure). Most residue analyses have been carried out with detection by UV absorption, and to a lesser extent by fluorescence or photoconductivity detection. The electrochemical detector is just beginning to find use in residue analysis. Refractive index detection has been reported infrequently, if at all. UV detection with a mercury lamp at its major emission wavelength of 254 nm is most often used, but use of the variable wavelength detector is growing because 254 nm or other wavelengths available from a mercury lamp are not optimal for many pesticides. Fluorescence detectors have been used in determining nonfluorescent pesticides by fluorogenic labeling employing derivatization methods similar to those applied earlier to facilitate thin-layer fluorodensitometry. The major disadvantage of HPLC at present is the poor sensitivity (ca. 10^{-7}-10^{-10}g) and selectivity of commercially available detectors. In order to improve sensitivity, interface devices have been developed to directly couple a liquid chromatograph with a mass spectrometer. A greater number of separations of greater complexity can be accomplished by HPLC than by GC since the mobile phase plays an active role in achieving resolution, and there is a wide range of stationary phases available for use in combination with a great variety of solvent mixtures and gradient elutions.

Quantitation of residues by scanning of thin layer chromatograms with commercial densitometers is widely applied for analysis of nonvolatile or unstable pesticides or where GC or HPLC equipment is not available. Precision, accuracy, and selectivity are often comparable to those techniques, and sensitivity is in the high pg-to-ng range for many analyses in which detection is made with fluorescence, chromogenic, or enzyme-inhibition reagents. For best quantitative results, sample applications are manually or automatically made to small areas on precoated, hard surface, high performance silica gel or reversed phase plates; detection reagents are uniformly applied by dipping rather than spraying; and samples and standards are developed together on each plate.

Other final determinative methods that have been applied to pesticide residues include polarography for compounds containing an oxidizable or reducible group, either naturally or after derivatization, atomic absorption, activation analysis, and radiochemical techniques. The latter are most often used in metabolism studies, for example thin layer chromatography of pesticides containing a radioactive isotope combined with autoradiography or radioscanning of the layers.

1E CONFIRMATORY TECHNIQUES

Three truly independent results are considered necessary for positive confirmation of the identity of a residue. Alternative methods that can be combined are TLC and/or paper chromatography with sorbent-solvent systems of different polarity or different visualization reagents, gas chromatography with columns of different polarity and selective detectors, preparation of derivatives to alter structure and volatility and thereby chromatographic properties, extraction p-values, ultraviolet photolysis, and mass spectrometry. Unlike conventional NMR, IR, UV, etc., mass spectrometry has sufficient sensitivity for general application to residue identification as well as for confirmation of pesticides in the presence of PCBs. Thus, the directly coupled gas chromatograph-mass spectrometer is a powerful tool for positive identification of mixture components at residue levels. The ability of the high resolution mass spectrometer to measure precise ionic masses has allowed individual pesticides with different elemental compositions to be identified in complex mixtures without prior separation in some cases.

The reliable detection and estimation of pesticide residues is one of the most difficult and demanding tasks an analytical chemist can be called upon to perform. Important commercial pesticides include insecticides, fungicides, herbicides, acaricides, and rodenticides. There are many hundreds of these compounds with greatly differing chemical structures and properties (e.g., organohalides, organophosphates, carbamates, anilines, ureas, phenols, triazines, quinones, etc.). Their determination may involve traces of any of these materials alone or in combination in a great variety of matrices, each with its own peculiar problems.

Further complications arise because metabolic degradation of certain pesticides produces compounds that may be more toxic and of different polarity than the parent pesticide. Examples include metabolically derived heptachlor epoxide and dieldrin, from heptachlor and aldrin, respectively, and oxygen analog metabolites of sulfur-containing organophosphorus pesticides. The analyst should be able to determine the identity and quantity of these metabolites and degradation products as well as the residue of the original pesticide, and extraction and cleanup procedures and chromatographic determinative conditions may have to be modified to accommodate these compounds. Multi-component pesticides such as chlordane, toxaphene, and strobane and their metabolites pose difficult confirmation and quantitation problems. Closely related, non-pesticidal compounds with similar analytical behavior such as PCBs or chlorinated naphthalenes may also be present in extracts, and the analyst must be able to isolate, identify, and measure pesticides of interest while simultaneously separating, isolating, and identifying these related compounds, if necessary. Trace contaminants contained in solvents or reagents, or extracted from plastic apparatus, can give rise to GC peaks or TLC spots that may be confused with pesticides. Positive confirmation of some pesticides is especially difficult because of very similar chromatographic properties of compounds, e.g., dieldrin and "photo-dieldrin."

The amount of effort expended and the choice of confirmatory tests are determined by the importance of the sample, resources available, and the amount of residue present. A possible alternative to testing of every residue is confirmation of selected samples at intervals, when the same residues are apparently present in all samples of a group. If sufficient residue is not available in individual members of a group of samples to permit use of a certain test, purified extracts are often pooled for confirmation.

1F AUTOMATION AND COMPUTER PROCESSING

Automation of pesticide analyses is presently in its early stages. Totally automated procedures have been developed for analyses not requiring column adsorption cleanup and those in which the final determination is colorimetry or UV absorption. Several microprocessor-controlled systems for automatic transfer of manually prepared samples onto a gas or liquid chromatography column are being marketed. Laboratories with high sample throughput can find such systems save time and cost in determinative steps. A system for automatic cleanup of samples by gel permeation chromatography has been designed, although automation of preparative and cleanup steps is not yet far advanced. Data systems allow storage of large amounts of data with computerized printouts that increase the speed and efficiency of analyses and improve both quantitation and identification of residues.

Although advances in automation are being reported at an ever-increasing rate, available systems are generally useful only for well-defined samples containing known pesticides. A skilled analyst using conventional, non-automated procedures is still required to carry out successfully multiresidue analyses of complex samples containing an unknown variety of pesticides and interferences. A proven, completely automated procedure for multiresidue analysis as it is usually performed (i.e., extraction, partition and adsorption chromatographic cleanup, and gas or liquid chromatography) is not yet available.

Since this introductory section is intended as a broad overview of modern residue analytical methods and their quality control, no details have been given. Much of the foregoing material will be discussed more completely in later sections, and specific references to relevant sections of the EPA Pesticide Analytical Manual or other sources will be given. A general bibliography of recent books and reviews on pesticide analysis follows.

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Section 2

INTERLABORATORY QUALITY ASSURANCE

2A QUALITY ASSURANCE PROGRAM OF THE EPA ENVIRONMENTAL TOXICOLOGY DIVISION (ETD) LABORATORY

Quality control in the context of this Manual connotes procedures taken to assure the accuracy and precision of analytical results. Qualitative and quantitative determinations by residue analysts are utilized for such important tasks as surveillance or monitoring of pesticide levels in human tissues, some segment of the environment, or the food supply; if conclusions and subsequent actions are to be valid, it is vital that the analytical data be reliable. The complex nature and pitfalls of the analytical procedures as outlined in Section 1 require a set of built-in controls to prevent or detect incorrect results. This Manual is dedicated to a program of quality control that will significantly minimize the output of unreliable and invalid analytical data. In a legal action, it is not unusual that the testimony of the analyst is evaluated on the strength or weakness of the operating quality control program in his laboratory.

The Quality Assurance Section of the Analytical Chemistry Branch, EPA-ETD Laboratory in Research Triangle Park, N.C., functions as the coordinating unit for a quality control program involving various laboratories in the EPA regions. This program was inaugurated in 1966 by the Technical Services Section of the Perrine Primate Laboratory, Perrine, FL., before the Laboratory was moved to North Carolina. Originally, the program was limited to Community Pesticide Studies, National Monitoring, and State Services Laboratories operating under contract with the U.S. Department of Health, Education, and Welfare, and more recently with the EPA to conduct chemical monitoring for pesticide residues in man and environment. Parts, such as the interlaboratory check sample program have now been expanded to include other state and private laboratories cooperating with the EPA.

The quality control program can be broadly divided into two classifications, both of which will be discussed in detail in this and the following Sections. The Interlaboratory control program, which was the first one formalized, involves analysis of uniform samples* by a number of participating laboratories in order to assess the continuing capability and relative performance of each. In addition, this program indicates, on a mathematical basis, the degree of confidence that can be placed in the results of sample analyses, and identifies analytical areas needing further attention. The coordinating laboratory receives data from the participating laboratories on a special report form, processes the data, ranks the laboratories in order of relative performance, and distributes the final results. Details of these procedures and typical sample data are given in Subsections 2D through 2J.

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*The terms "check sample" and "blind sample" are used interchangeably for the samples prepared and distributed by the coordinating laboratory. The former term should not be confused with the other widely used meaning of "check sample" (or control sample), that is a sample substrate known to initially contain no pesticides, and then spiked to evaluate recovery by a certain procedure.

The Intralaboratory control program, which will be treated in detail in Section 3, assists a single laboratory in improving the accuracy and precision of data produced by its personnel by providing systematic guidelines for top quality analytical methodology and techniques. One feature of this program is the continual, periodic analysis of standard reference materials (SPRM's) by each analyst and recording of the results on a graphical quality control chart. This chart, which is a plot of the analytical results vs. their time or sequence, evaluates periodic performance in terms of both precision and accuracy and includes upper and lower control limits to serve as criteria for remedial action or for judging the significance of variations between duplicate samples.

A "Statistical Terms and Calculations" subsection at the end of Section 2 will explain some basic terms, equations, and operations used in the quality control programs for data handling and calculation and statistical evaluation of analytical results.

2B PROGRAM OBJECTIVES

The objectives of the interlaboratory program are:

a. To provide a measure of the precision and accuracy potential of analytical methods run routinely by different laboratories.

b. To measure the precision and accuracy of results between laboratories.

c. To identify weak methodology.

d. To detect training needs.

e. To upgrade the overall quality of laboratory performance.

2C PROGRAM ACTIVITIES

The interlaboratory program includes the following activities:

a. Analysis of interlaboratory check samples by all participants.

b. Operation of a repository to provide any non-profit laboratory with analytical grade pesticide reference standards, over 700 of which are now available. These are listed in an index available from the ETD laboratory.

c. Providing uniform, standard analytical methods in the form of an analytical manual also available from the ETD laboratory.

d. Quality control of materials of uniform standard quality such as precoated GC column packings, cleanup adsorbent, etc. These materials are purchased from commercial suppliers under stringent specifications in bulk lots, and distributed in individual units to EPA laboratories and other facilities under formal contract with EPA to conduct pesticide studies.

e. Providing abbreviated, informal, on-the-job training for specific requirements.

f. Assisting with problems relating to analytical methodology by phone, mail, or on-site consultations.

g. Operation of an electronic facility for repair, overhaul, design and calibration of laboratory instruments.

2D TYPES AND PREPARATION OF SAMPLE MEDIA

The check sample program is probably the most important interlaboratory activity because all allied activities closely depend on it. Samples used in the program are mixtures of pesticides in a substrate ranging from pure solvent, in the simplest case, to those media routinely analyzed by the participating laboratories, such as fat, blood serum, gonad, brain, and liver tissue, water, soil, and simulated air samples.

As an example, a description is given of the preparation and handling of a blood interlaboratory check sample by the coordinating laboratory: General population serum samples are obtained from a local blood bank, typically in 300 ml bottles. The frozen samples are thawed in a refrigerator (2-3°C), poured together into a stainless steel container (previously rinsed with acetone), and mixed well. Approximately 4 liters of serum have been sufficient for the program for one year. Experienced chemists analyze the pooled serum to establish the base level profile and to be sure no gross contamination is present. Part of the sample is then divided into small storage bottles with Teflon-lined caps and stored in a freezer (-18 to -23°C). The remainder is stored in bulk in the freezer for later spiking.

Sub-samples are mailed to participating laboratories to serve as their interlaboratory check sample and to provide sufficient intralaboratory standard pesticide reference material (SPRM) for six months. Each laboratory supervisor requests in advance the amount of sample required for the latter purpose based on his estimated routine sample load (see Subsection 3D). A careful study has indicated there is no need to mail the samples frozen because neither pesticide nor sample degradation has been observed in a 3-to 4-day period. After removing the amount required for the interlaboratory check sample, personnel at each laboratory sub-divide the remainder into small vials that are stored continuously in a freezer. Individual vials are removed as needed to provide 2.0 ml intralaboratory SPRM samples.

The next time an interlaboratory blood sample is required, the same pooled base sample is spiked with pesticides common to blood. This sample will allow the participating laboratories to test their recoveries at high pesticide levels, thereby simulating analysis of routine samples from individuals occupationally exposed to high pesticide levels. Again, enough sample will be provided each laboratory to serve both as interlaboratory sample and intralaboratory SPRM's for six months.

The same basic procedure is used for other check sample substrates. Rendered chicken fat from a poultry plant has been used for fat samples, while animal brain, gonad, and other tissue check samples have also been prepared. It is anticipated that urine, milk, and soil samples for testing certain procedures will be supplied in the future.

With the check sample, each participant receives a covering letter providing the protocol for handling the sample. The time allowed for analyzing and reporting results corresponds to the normal time for processing a similar routine sample.

Although it is presumably a blind sample to be analyzed along with the daily work load, the interlaboratory check sample will most often be recognized as such by the chemist at the time of analysis. The chemist is likely to give special care and attention to this sample, and, in addition the best chemist in the laboratory may be assigned the sample in the first place. Therefore, poor results on an interlaboratory check sample must be considered a serious matter since they will often represent the very best work the laboratory produces.

The importance of the interlaboratory check sample program is indicated by a number of actions that were initiated toward standardization based on information obtained over the years. These include distribution of pretested Florisil cleanup adsorbent and GC column packings and frequently updated standard analytical methods, and a centralized calibration and electronic repair facility.

2E REPORTING FORMS

Laboratories are requested to report their results on special forms. The forms are designed to provide supplemental operating data in addition to numerical results of the analysis. The standard reporting form, with detailed instructions for completion on the reverse side, is shown as Table 2-1. The data and information supplied by each laboratory include the sample size, extent of concentration of the sample extract, injection volumes, elution cuts if column cleanup is required, all instrumental operating parameters, identity of the GC column, and the numerical data and original chromatograms upon which all calculations are based. The chromatograms must be clearly identified so that they may be related to the data on the reporting forms for easy checking of the quantitative results by the coordinating laboratory.

2F EVALUATION OF REPORTED DATA

When the completed reporting forms from the participating laboratories are received in the coordinating laboratory, the quantitative results are entered on a Summary of Results sheet illustrated in the next subsection. If any results appear obviously and grossly erroneous, the laboratory is contacted at once and given a chance to review its work and change the report if a simple computational or transcription error is found. After all results are recorded, a statistical analysis of the results is made and recorded on the Summary of Results sheet. A relative performance or ranking table is also prepared, establishing a numerical ranking value for each laboratory (Subsection 2H).

After the data evaluations and calculations are made, the completed report forms and chromatograms from the laboratories with the poorer rankings are subjected to detailed examination to determine, if possible, the reasons for the inferior performance. Availability of the actual recorder traces of the chromatograms for study is vital because they allow the coordinating laboratory to check such factors as column efficiency, sensitivity of detection, instrumental problems such as baseline noise and improperly adjusted recorder gain, proper operating parameters to produce well-resolved peaks, inaccurate reference standards, and faulty quantitation procedures. A detailed critique is then written, and in cases of extremely poor performance, the laboratory is immediately contacted by phone to apprise it of the poor ranking and to make suggestions to improve its performance.

Table 2-1

REPORT OF INTERLABORATORY CHECK SAMPLE

(Circle Where Appropriate)

Date ____________________

Lab. Code No. ______ **Sample No.** ______ **Origin. Sample Size** ______

GC COLUMN AND INSTRUMENTAL DATA

COLUMN

Identity: ____%______/____%________ Inside Diam. ________

Length ______ Carrier Gas ______, Flow Rate ______ ml/min.

TEMPERATURES, °C

Inlet ______, Col. Oven ________

Transf. Line ______ Detect. ________

DETECTOR

Electr. Capt.: ^{63}Ni Other Direct DC Pulsed Linearized

Flame Photomet.: P or S, Gas Flow, ml/min. H_2 ___ O_2 ___ Air ______

GC RESPONSE: ______% F.S.D. for ______ pg Aldrin or ______ ng E. Parathion at Attenuation of ______________

RECORDER: Chart Speed ________ in./min.

Compound	Sample Extract Data						Reference Standard Data				Results				Chromat. No.
	Gms in Final Vol.	Ml in Final Vol.	µl Inject.	mg Inject.	Peak Ht. or Area	Attenuat	µl Inject.	ng Inject.	Peak Ht. or Area	Attenuat.	RRT Relative to		Residue		
											Sample	Stand.	ppm	ppb	

1. Source of Method (Cite journal, volume, page, year): ______________________ Modifications if any: ______________________

2. Extract Concentration (circle) Air Blowdown N_2 Blowdown Kuderna-Danish

3. Simple Boiling Roto-Vac Other (describe) ______________________

4. Refer to back for instructions for completion of this report

____
Signature of Chemist Doing Work

INSTRUCTIONS FOR COMPLETION OF REPORT

1. If we are to provide you with a critique on the analytical performance the data requested on the report form must be complete. All of it is meaningful for a full performance evaluation whether it makes sense to you or not.

2. Use one report form for each GLC column and show under RESIDUE only those values you want to appear in the final summary of results. For example, if a given compound is quantitated on two columns, report only the value in which you have most confidence. Do not leave the choice to us.

3. Under the column headed Ml in Final Vol., the numerical value to be placed here should be the final volume after concentration (or dilution). For example, in handling a blood sample, an extract concentration down to 1.0 ml might prove necessary for the quantitation of dieldrin. In this case, the figure 1.0 would appear in the column opposite dieldrin. However, for the determination of p,p'-DDE, a dilution of the concentrated extract up to 10 ml may be indicated. In this case, the figure 10 would appear opposite p,p'-DDE.

4. Each chromatogram of sample and standard shall be sequentially numbered. These numbers are then to be written on the appropriate lines in the column headed CHROMATOGRAM NUMBER. Two numbers should then appear opposite each pesticide reported, one representing the standard chromatogram, the other representing the sample.

5. Include with the chromatograms a standing current profile for each E.C. detector being used in D.C. mode. Label each step with the polarizing voltage for that step.

6. Mail only original chromatograms with your report, not photocopies. All chromatograms will be returned. Fold chromatograms for each column in accordion fashion from one continuous roll. Do not slice up. If your recorder runs a considerable distance beyond the last peak, don't slice it off right after the peak. Let us have the whole pen run.

7. With your report, include all chromatograms related to the sample work whether used for quantitation or confirmation. However, for those G.C. columns used for confirmation only, include all data on the report form except a final quantitative value.

8. On submitted chromatograms, identify each peak resulting from a standard injection and show the amount of compound represented by the peak in pg or ng.

The reports from all other laboratories are then scanned to locate any irregularities that may lead to future problems. A general letter is drafted, and a copy is mailed to all participating laboratories. The letter discusses common analytical difficulties encountered by several laboratories and offers suggestions that appear to have general applicability for improving compound identification and quantitation. Each laboratory also receives a copy of the Summary of Results, with each laboratory identified by a code number, and a copy of the Relative Performance Table. Finally, a private critique of performance is sent to each laboratory exhibiting special need for help (Subsection 2I).

2G SUMMARY OF RESULTS TABLES AND RESULTS OF CHECK SAMPLE ANALYSES

Typical summary tables are illustrated as Tables 2-2 through 2-13. Definitions and means of calculating the items included in the statistical report at the bottom of each table are given in Subsection 2K.

TABLE 2-2

INTERLABORATORY CHECK SAMPLE NO. 26, MIXTURE OF STANDARDS IN SOLVENT

SUMMARY OF RESULTS.

LAB CODE NUMBER	PESTICIDES REPORTED IN PICOGRAMS PER MICROLITER														
	Lindane	Aldrin	Hept. Epoxide	p,p'-DDE	Dieldrin	Endrin	o,p'-DDT	p,p'-DDT	Perthane	Ethion	o,p'-DDD	p,p'-DDD	Mirex	DCPA	β-BHC
	10	10	10	75	20	30	20	100	0	0	0	0	0	0	0
45.	12	46*	53*	379*	145*	87*	32	578*							
47.	10	11	11	88	---	---	---	195*							
48.	9.7	9.2	8.7	73	31	29	24	113							
52.	18*	11	14	29*	16	15	---	---	379						
53.	14	14	14	47	18	87*	---	91							
54.	29*	3.0*	6.0*	---	---	---	---	22*	34			4.0	29	9.0	
66.	9.4	8.0	9.2	66	14	30	14	115							
68.	10.1	10	8.5	76	21.6	---	65*	99							
69.	10.4	3*	---	---	92*	---	24	---							
71.	10	5.0	10	---	100*	60*	---	150*							
72.	13.8	8.8	9.4	91	19.5	42	20	98							
73.	12	12	10	64	19	27	33	94							
83.	11.4	14.1	13.5	59	29	39	32.5	88							
84.	8.5	9.6	8.4	70	10	15	11	94							
85.	8.5	8.3	8.1	75	18	27	20	90							
87.	9.5	10.6	9.6	63	22.8	32	24	99							
88.	4.0*	14	12	78	22	12	28	94							
89.	9.0	8.0	10	70	10	14	23	120							
90.	4.8*	6.9	5.1*	45	11.5	---	41	73							
92.	9.0	10	9.0	90	20	---	46	115							
93.	10	12	81*	22*	32	33	31	125				16			
95.	11	8.0	8.0	80	21	---	---	87							
96.	12.4	12.1	10.9	119*	21.7	35.5	27.6	150*							
97.	9.4	11	9.8	89	23.4	33.6	41	110				13.2			2.5
113.	9.0	8	8.6	69	16.4	29	20.4	94							
113A.	9.2	7.8	9.4	72	16.8	28	20.4	96							
130.	11.2	11.0	11.0	86	23.8	29	26.5	112							
135.	9.8	9.4	9.3	74	14	25	21	104							
137.	11	10	10	73	23	29	27	103							
160.	11	9.0	10	102	---	24	---	97		68					
161.	9.7	9.6	10.4	70	16.9	28	18.5	95							
162.	10.1	12.2	10	77	24.3	36	22.6	101							
163.	14	11	10	78	19	--	36	96				12			
164.	4.5*	4.8	6.3*	18*	13.4	24.9	16.2	56*			20				
Mean	10.5	9.9	10.1	74.0	19.6	27.7	26.2	100							
Std.Dev.	1.56	2.31	1.61	12.9	5.64	7.82	8.54	11.6							
Rel.Std. Dev.,%	14.9	23.4	15.9	17.4	28.8	28.2	32.6	11.6							
Total Error,%	36	4.7	33	36	58	60	116	23						*Rejected as outliers	

TABLE 2-3

INTERLABORATORY CHECK SAMPLE NO. 26, MIXTURE OF STANDARDS IN SOLVENT

SUMMARY OF RESULTS

LABORATORY CODE NO.	PESTICIDES REPORTED IN PICOGRAMS PER MICROLITER								
	Lindane	Aldrin	Hept. Epoxide	p,p'-DDE	Dieldrin	Endrin	o,p'-DDT	p,p'-DDT	p,p'-DDD
	10	10	10	75	20	15	20	100	0
1.	9.7	10.3	9.3	71	19.7	14.2	23.7	94	
2.	10	9.9	9.7	75	18.9	13.2	24.7	99	
3.	9.9	10	10	75	18	13	21	83*	12
4.	8.5	11.3	10.4	102*	22.6	15.9	30.4	99	
5.	10.1	9.7	10.4	81	15.3	10.0*	24.2	99	
6.	10.7	9.9	10.2	77	20.2	14.8	27.6	102	
7.	9.5	9.7	9.9	75	21	15.2	21.8	100	
8.	9.8	9.9	8.9	75.5	15.7	15.4	23.8	99	
9.	10	9.0	9.5	72	19.3	17	23	100	
10.	10.2	9.5	9.8	74	20.2	18	26.4	111	
11.	10.3	10.1	10.3	83	18	13	25	100	
12.	10	10.3	10.6	84	20	11.7	23	99	
13.	9.3	8.9	9.2	82	19	14	22	91	
14.[1]	6.8*	7.2*	7.0*	56*	17.6	13.2	19.5	83*	
1/ Lab recently joined program.									
Mean	9.9	9.9	9.9	77	19	14.5	24	99.4	*Rejected as outliers
Std. Deviation	0.54	0.60	0.52	4.37	1.96	1.78	2.79	4.70	
Rel. Std. Dev. %	5.5	6.1	5.3	5.7	10.3	12.3	11.6	4.7	
Total Error %	12	13	11	14	25	27	48	10	

TABLE 2-4

INTERLABORATORY CHECK SAMPLE NO. 25, SERUM

SUMMARY OF RESULTS

LABORATORY CODE NO.	PESTICIDES REPORTED IN PARTS PER BILLION					
	Dieldrin	o,p'-DDT	p,p'-DDE	p,p'-DDT	β-BHC	HCB
	4.0	6.0	45	8.0	0	0
1.	2.2*	5.0	46	8.7		
2.	5.0	5.1	42	9.8		
3.	4.0	5.0	41	10		<1
4.	6.0*	7.9	62*	14*	1.9	
5.	3.7	6.0	39	9.6		
6.	4.2	6.5	53	9.3		
7.	3.5	6.5	50	9.8		
8.	3.9	5.4	46	9.2		
9.	3.8	5.6	44	10		
10.	4.0	4.0	35	8	1.0	
11.	4.1	5.0	44	8.3		
12.	3.9	5.8	39	9.3	1.3	
13.	3.4	4.6	52	7.3		
14.	4.5	5.2	44	8.7		
15.	4.1	4.9	38	9.8		
16.	3.6	5.5	44	9.6		
17.	4.9	5.1	39	8.7		
18.	4.4	8.4	38	10.8		
Mean	4.1	5.6	41	9.2		*Rejected as outliers.
Std. Deviation	0.46	1.11	5.12	0.86		
Rel. Std. Dev. %	11.2	19.7	12.5	9.4		
Total Error %	25	44	24	37		

Table 2-5

BLOOD SERUM, INTERLABORATORY CHECK SAMPLE NO. 33 (1975)

Lab. Code No.	PESTICIDES REPORTED IN PARTS PER BILLION					
	Dieldrin	o,p'-DDT	p,p'-DDE	p,p'DDT	Epoxide	β-BHC
	6.00^a	3.8^a	30^a	13.7^a	--	--
1	5.70	--	26	9.8		
10 No sample issued						
8	5.60	2.78	28	11.1	p,p'-DDD	
12	5.20	2.50	22	10.8	1.4	1.3
7	5.90	3.30	28	11.6	Tr.	Tr.
14	6.10	3.80	23	11.0		
15	5.40	2.90	30	14.0		
25	6.00	3.00	26	9.0		
5	6.75	3.65	28	13.0		
26	6.40	4.50	31	14.7		1.00
4 No blood analyt. work						
24	5.50	3.50	29	11.9		
11 No report rec'd.						
6 (First run)	6.00	4.00	21	12.0		
29	5.46	3.06	23	4.3*		
Overall Mean	5.83	3.36	26.3	11.7		
Std Dev	0.45	0.59	3.30	1.69		
Rel Std Dev, %	7.67	17.6	12.6	14.5		
Total Error, %	17.7	42.7	34.5	39.2		

a. Parts per billion present. Values approximate.
* Rejected as an outlier.

Table 2-6

INTERLABORATORY CHECK SAMPLE NO. 40, SERUM - SUMMARY OF RESULTS (1976)

Lab. Code No.	PESTICIDES REPORTED IN PARTS PER BILLION								
	Dieldrin	o,p'-DDT	p,p'-DDE	p,p'-DDT	Trans-Nonachlor				
	4.0[a]	3.0[a]	28.0[a]	5.0[a]					
1	3.8	3.3	24.5	5.4					
4	4.2	4.5	26.7	4.3					
5	4.2	3.9	26.8	5.0					
7	3.8	2.5	27.1	4.6					
8	3.2	3.3	24.1	3.9					
10	3.9	4.5	25.9	5.0					
11	2.8	3.7	24	5.0					
12	4.3	3.4	22.9	5.6					
13	4.6	5.5	24.0	5.2					
14	5.0	5.1	22	6.4					
15	6.0	3.8	29	4.8					
16	3.9	2.8	23.8	6.2					
24	Results not received in time to include								
25	4.1	3.1	34	4.4	1.0				
26	4.2	4.8	28.5	5.7					
Overall Mean	4.1	3.9	26.0	5.1					
Std Dev %	0.7583	0.8922	3.112	0.7098					
Rel Std Dev %	18.5	22.9	12.0	13.9					

[a] Parts per billion present. Values approximate.

Table 2-7 INTERLABORATORY CHECK SAMPLE NO. 54, BLOOD SERUM - SUMMARY OF RESULTS (1978)

Lab. Code No.	PESTICIDES REPORTED IN PARTS PER BILLION									
	p,p'-DDE	p,p'-DDT	HCB	Trans-Nonachlor	PCP	o,p'-DDT	β-BHC	Hept. Epoxide	Dieldrin	p,p'-DDD
	0[a]	0[a]	10[a]	5[a]	50[a]					
4	19.3	2.85	10.2	5.8	190					
5	15.6	7.1*	8.8	5.7						
7	22.8	--	16.6*	4.4	102					
8	18.0	2.40	10.0	4.4						
10	19.9	1.40	9.5	5.1						
11	16.2	2.49	8.8	4.5		1.48				
12	15.8	2.4	9.7	6.5						
14	19.0	4.5	11.0	5.2			1.2			
15	17.0	2.2	13.9	--				4.1		
24	18.3	2.5	9.5	5.6						
25	18.2	2.5	11.8	5.6					1.20	
26	18.3	2.23	8.4	5.9	142					
1	20.0	3.7	9.4	8.2*						
9	11.9	--	10.5	5.4						
13	17.5	2.3	9.9	5.7						
16	28.5*	1.78	7.6	6.9				1.3	0.5	1.3
Overall Mean	17.9	2.56	9.93	5.48						
Std Dev, %	2.47	0.79	1.51	0.733						
Rel Std Dev, %	13.8	31	15.2	13.4						

[a] These values represent only spike added.

* Rejected as outliers.

Table 2-8

INTERLABORATORY CHECK SAMPLE NO. 59, SERUM - SUMMARY OF RESULTS (1978)

Lab. Code No.	PESTICIDES REPORTED IN PARTS PER BILLION						
	β-BHC	Hept. Epoxide	Trans-Nonachlor	Dieldrin	p,p'-DDE	p,p'-DDT	
	7^a	5^a	8^a	8^a	-	4^a	
4	7.49	4.32	8.61	7.59	24.6	5.43	1.00-p,p'-DDD
5	7.36	5.22	6.72	8.55	13.2	3.48	
7	5.98	5.31	5.09	6.79	13.14	4.33	
8	7.39	5.87	8.05	8.52	17.45	5.87	
11	7.96	5.06	7.24	8.83	12.84	6.07	
12	8.85	8.40	10.11	9.68	18.87	6.21	
14	7.2	5.6	6.9	10.0	18.0	6.8	
15	7.2	5.1	6.9	8.9	18.9	5.1	
25	6.86	3.78	7.42	8.39	18.11	5.61	
26	5.81	4.84	8.18	7.74	15.13	4.91	
1	6	4.1	7.0	6.6	14.1	5.0	
6	4.8	4.5	8.2	5.3	14	4.1	
9	--	5.15	11.44	8.87	20.02	6.54	0.62-Lindane
13	5.92	4.47	5.6	7.57	10.60	6.45	
16	8.44	5.98	9.91	9.25	26.42	5.30	
32	6.49	3.50	6.22	5.29	11.0	7.69	
34	1.67	2.13	1.44	1.80	2.13	--	
Mean	6.92	4.68	7.72	7.99	16.65	5.56	
Std Dev	1.09	0.98	1.68	1.41	4.53	1.08	
Rel Std Dev, %	15.8	21.0	21.8	17.6	27.2	19.5	
Total Error, %	32.3	45.6	45.5	35.4	56.6	39.0	

[a] Parts per billion present.

Table 2-9

INTERLABORATORY CHECK SAMPLE NO. 66, SERUM - SUMMARY OF RESULTS (1979)

Lab. Code No.	PESTICIDES REPORTED IN PARTS PER BILLION							
	Oxy-chlordane	Hept. Epoxide	Trans-Nonachlor	p,p'-DDE	p,p'-DDT	Mirex		
	7.5^a	9.4^a	9.5^a	--	7.0^a	10.0^a		
4	7.61	7.90	8.70	14.1	6.00	--		
5	7.36	10.3	7.88	10.0	3.48*	--		
7	7.8	11.0	11.1	14.0	5.5	--		
8	6.90	7.95	7.96	14.33	6.76	<10		
11	5.99	8.23	6.64	9.96	--	--		
12	6.94	9.60	9.77	15.10	7.84	--		
14	5.6	8.5	7.3	12.0	6.8	--		
24	6.52	8.22	8.43	12.55	7.55	2.1		
25	7.54	9.04	9.68	13.69	5.56	--		
26	7.35	7.50	8.29	12.50	6.0	--		
1	8.7	10.4	9.0	16.6	5.0	--		
6	5.8	7.6	6.8	11.0	5.2	--		
13	7.47	10.44	6.42	9.65	6.17	--		
16	6.16	6.74	6.18	10.28	5.95	--		
38	6.58	8.22	8.34	14.03	5.68	--		
52	7.4	9.4	9.5	14.5	7.4	2.1		
Mean	6.98	8.81	8.25	12.77	6.24			
Std Dev	0.83	1.25	1.35	2.10	0.89			
Rel Std Dev, %	11.91	14.13	16.65	16.48	14.29			
Total Error, %	29.1	32.7	42.1	39.7	36.3			

[a] Parts per billion present.
*Rejected as an outlier

Table 2-10

COLLABORATIVE SAMPLE NO. 21, SPIKED FAT

SUMMARY OF RESULTS

Lab. Code No.	PESTICIDES REPORTED IN PARTS PER MILLION							
	Aldrin	Hept. Epoxide	Dieldrin	o,p'-DDT	p,p'-DDD	p,p'-DDE	p,p'-DDT	β-BHC
	0.10	0.30	0.70	0.80	0.40	9.0	3.0	0
1	--	0.33	0.45	0.76	0.37	8.70	3.11	
4	0.090	0.27	0.58	0.63	0.44	8.66	2.63	
5	0.13	0.34	0.60	1.02	0.47	7.52	2.09	
6	--	0.26	0.67	0.64	0.42	9.32	2.86	
7	0.090	0.30	0.75	0.74	0.48	7.5	2.60	
8	0.090	0.31	0.71	0.75	0.48	8.80	3.08	
9	0.070	.060*	0.54	0.67	0.44	7.10	2.36	
11	0.060	0.27	0.050*	0.70	0.29	7.23	2.90	
14	--	0.32	0.52	0.80	0.53	8.51	2.67	
15	0.10	0.30	0.80	0.68	0.37	8.90	3.10	
16	0.080	0.31	0.70	0.73	0.40	8.29	3.03	
24	0.060	0.020*	0.39	0.43*	0.32	6.70	1.60*	0.020
25	0.090	0.31	0.69	0.72	0.48	9.32	2.70	
26	0.080	0.22	0.63	0.53	0.33	6.59	3.12	
31	0.050	0.25	0.33	0.50	0.24	6.30	2.20	
33	0.060	0.25	1.12*	0.67	0.32	7.56	2.71	
34	0.060	0.19	1.12*	0.52	0.39	9.07	3.53	
Mean	0.080	0.28	0.60	0.68	0.40	8.03	2.73	
Std Dev	0.022	0.042	0.14	0.12	0.080	1.00	0.38	
Rel Std Dev, %	28	15	23	18	20	12	14	
Total Error, %	65	35	54	46	40	33	34	

[a] Parts per million present.

* Rejected as outliers.

Table 2-11

INTERLABORATORY CHECK SAMPLE NO. 56, FAT--SUMMARY OF RESULTS (1978)

Lab. Code No.	PESTICIDES REPORTED IN PARTS PER MILLION							
	HCB	Aldrin	Oxychlordane	Hept. Epoxide	Trans-Nonachlor	p,p'-DDE	p,p'-DDT	
	0.050[a]	0.20[a]	0.25[a]	0.20[a]	0.15[a]	1.50[a]	0.50[a]	
4	0.027	0.15	0.222	0.193	0.135	1.48	0.39	dieldrin--0.01
7	0.0392	0.204	0.200	0.309	0.112	1.81	0.586	
8	0.030	0.16	0.24	0.16	0.11	1.35	0.49	dieldrin--0.01, β-BHC--<0.02
11	0.058	0.223	0.316	0.261	0.183	1.54	0.506	dieldrin--0.006, Thiodane I--0.004
12	0.031	0.157	0.23	0.226	0.174	1.33	0.421	
14	0.028	0.143	0.251	0.166	0.109	1.40	0.434	dieldrin--0.005
15	0.04	0.18	0.26	0.20	0.15	1.46	0.52	
25	0.036	0.154	0.239	0.175	0.146	1.26	0.490	dieldrin--0.009
26	0.022	0.098*	0.185	0.184	0.133	1.17	0.492	
1	0.04	0.20	0.25	0.19	0.16	1.20	0.40	
6	0.052	0.19	0.055*	0.18	0.14	1.70	0.51	
13	0.021	0.135	0.205	0.157	0.118	1.13	0.49	
16	0.027	0.189	0.209	0.024*	0.106	2.81*	0.474	
31	0.25*	0.176	0.297	0.252	0.128	1.05	0.675*	
34	0.068	0.167	0.283	0.22	0.18	1.01	0.439	
38	0.050	0.18	0.224	0.211	0.158	1.44	0.443	
Overall Mean								
Std Dev								
Rel Std Dev, %								
Total Error, %								

[a] Parts per million present.

*Rejected as outliers.

Table 2-12

INTERLABORATORY CHECK SAMPLE NO. 70, FAT--SUMMARY OF RESULTS (1979)

Lab. Code No.	PESTICIDES REPORTED IN PARTS PER MILLION									
	HCB	β-BHC	Oxychlordane	Trans-Nonachlor	Hept. Epoxide	p,p'-DDE	p,p'-DDT	Dieldrin	Aroclor 1254	Non-Spike
	0.061[a]	0.25[a]	0.10[a]	0.15[a]	0.81[a]	3.50[a]	0.60[a]	0.13[a]	1.00[a]	
4	0.038	0.278	0.114	0.156	0.092	3.39	0.675	0.162	1.99*	
7	0.035	0.269	0.079	0.175	0.091	3.289	0.705	0.149	+	o,p'-DDT = 0.057
8	0.038	0.22	0.10	0.16	0.105	3.31	0.59	0.133	1.40	
10	0.041	0.244	0.094	0.154	0.095	3.212	0.581	0.081	--	o,p'-DDT 0.062 Aldrin 0.014
11	0.032	0.23	0.075	0.12	0.086	3.3	0.68	0.13	--	
12	0.042	0.278	0.117	0.148	0.096	2.148	0.741	0.132	--	Aldrin 0.018
14	0.032	0.234	0.057	0.151	0.062	2.097	0.481	0.082	--	
24	0.049	0.180	0.074	0.130	0.080	2.40	0.470	--	0.505	o,p'-DDT 0.052
25	0.042	0.258	0.105	0.152	0.090	3.964	0.559	0.152	1.328	o,p'-DDT 0.040 p,p'-DDD 0.045
26	0.022	0.237	0.105	0.13	0.059	2.96	0.588	0.148	0.943	
51	0.04	0.25	0.09	0.15	0.10	3.10	0.60	0.13	0.96	
1	0.06	--	0.16	0.11	0.03	3.36	0.80	0.13	--	o,p'-DDT 0.14 Ald. 0.02 Lindane 0.05
6	0.048	--	--	0.12	0.064	3.40	0.58	0.12	1.00	
9	0.042	0.264	0.182*	0.208*	0.086	2.577	0.819	0.102	--	
13	0.024	0.242	0.083	0.143	0.094	2.061	0.575	0.029	--	
16	0.045	0.212	0.122	0.165	0.073	2.98	1.01*	0.124	--	
38	0.043	0.223	0.058	0.116	0.053	2.992	0.503	0.118	1.00	
52	0.014*	0.119*	0.036	0.115	0.015*	2.166	0.403	0.019*	0.519	
Overall Mean	0.049	0.24	0.092	0.14	0.080	2.93	0.61	0.12	0.96	
Std Dev	0.009	0.03	0.030	0.02	0.02	0.56	0.12	0.03	0.32	
Rel Std Dev, %	23.0	11.0	32.6	14.0	25.1	19.0	18.9	27.7	33.8	
Total Error, %	65.0	24.8	68.0	32.4	51.1	48.1	39.9	58.8	69.1	
Av % Recovery	66.7	96.0	92.0	93.3	98.8	83.7	101.7	92.3	96.0	

[a] Parts per million present
*Rejected as an outlier

Table 2-13

INTERLABORATORY CHECK SAMPLE NO. 49, WATER--SUMMARY OF RESULTS

	PESTICIDES REPORTED IN MICROGRAMS PER LITER (OR PARTS PER BILLION)					
Lab. Code No.	HCB	Oxychlor-dane	p,p'-DDE	p,p'-DDT	Aroclor 1254	
	0.30[a]	0.40[a]	0.60[a]	1.60[a]	10[a]	
8	0.20	0.30	0.40	1.30	9.1	
166	--	45.*	81.0*	151.*	627.*	
15	0.18	0.33	0.53	1.50	10.9	
6	--	--	--	--	7.9	
7	0.20	--	--	2.12	13.4	
11	0.13	0.31	0.43	1.20	5.6	
34	0.14	0.17	0.23*	0.97	5.1	
9	0.18	0.40	0.50	1.18	--	p,p'-DDD---0.47, trans-Nonachlor---0.28 Kepone---3.3, o,p'-DDT--0.23
36	0.18	0.65*	0.53	1.60	7.5	o,p'-DDT--0.15
26	0.20	0.30	--	--	8.6	
13	0.26	0.36	--	--	3.9	
3	0.25	0.36	0.47	1.51	8.4	
23	--	--	0.83	2.68*	--	o,p'-DDT--0.45, Hept. Epoxide--0.42 o,p'-DDE--0.69, Dieldrin--0.96
1	0.25	--	--	-	10	
24	0.16	--	--	--	16.7*	
25	0.22	0.44	0.56	1.41	7.3	
5	0.24	0.36	0.56	1.50	6.8	
12	--	--	0.45	1.20	--	Aldrin--0.04
10	--	--	0.35	1.00	8.8	Aroclor 1248--13.4
Overall Mean	0.20	0.33	0.51	1.37	8.09	
Std Dev	0.04	0.07	0.13	0.31	2.43	
Rel Std Dev, %	20	22	26	23	30	
Total Error, %	50	53	58	53	68	

a. Parts per billion present.

b. Reporting units questioned and verified.

* Rejected as outliers.

a. Check Samples Composed of Standards Dissolved in Solvent

Table 2-2 shows data from a group of 34 laboratories participating in an interlaboratory control program for the first time as a group entity. The distributed sample consisted of a precise formulation of eight chlorinated pesticide and metabolite standards dissolved in pure solvent in a sealed ampoule; no cleanup steps were required. The mean and standard deviation values were calculated after rejection of the outlying values designated by asterisks. (See Subsection 2Kf for description of fitness test). The precision (relative standard deviation) was considered "good" for this type sample only for the compounds lindane, heptachlor epoxide, and p,p'-DDT, "fair" for p,p'-DDE, and "poor" for the other four. The overall average RSD (relative standard deviation; Section 2Ke) for all compounds was 21.6%, nearly double the value expected from a group of laboratories with top quality analytical output, such as illustrated by Table 2-3. Total error values considered "good" include lindane, heptachlor epoxide, and p,p'-DDT, aldrin is "fair", and the others "poor." The average total error was 52%, just outside the "acceptable" level of <50%.

Table 2-3 shows, for comparison, results on the same sample (except for a more difficult, lower endrin content) by a group of laboratories that (except for one) had been in the quality control program for several years. The calculated average RSD value is 7.7% and the average total error is 20%, both "excellent" performance values. The average total time spent in each laboratory on the sample by this group was 1.5 days. During the earlier years of participation, the data output of these laboratories was similar to that shown in Table 2-2, but continuing participation in both Inter- and Intralaboratory Programs resulted in gradual improvement in performance to the levels shown in Table 2-3. As an example of a factor responsible for the poor results in Table 2-2, the 34 laboratories used 33 different GC columns, while the experienced group represented in Table 2-3 used only the optimum GC columns and the operating parameters recommended in the EPA Pesticide Analytical Manual and in Section 5 of this Manual.

b. Blood Serum Check Samples

Table 2-4 shows results for a blood serum check sample reported by 18 laboratories with experience in the quality control program. The average RSD of 13% and total error of 33% are quite acceptable for this type of sample.

Table 2-5 shows results for a second serum check sample reported by 12 participating laboratories. This sample was prepared from the same base lot of serum used for a previous sample (No. 31) issued earlier. It was held in a deep freeze for the intervening six months. The formulation values on the summary sheet were regarded as approximate. The formulation was based on the data profile on the sample as it was originally received, plus data from the laboratories analyzing the earlier check sample. The values were believed to be valid within ca ± 15%. Precision on this sample is very good for dieldrin, p,p'-DDE, and p,p'-DDT and is fair for o,p'-DDT. The total error, embracing both precision and accuracy, is highly satisfactory for dieldrin and satisfactory for the other three spiking compounds. Two laboratories reported traces of heptachlor epoxide and β-BHC, both of which compounds were probably actually present in trace quantities.

Results of a third serum check sample are given in Table 2-6. The mean values reported are very close for dieldrin and p,p'-DDT (102% recovery in each case), high for o,p'-DDT (130% recovery), and slightly low for p,p'-DDE (93% recovery). No reported values were rejected, but the laboratories with DDE values below 23 ppb and the one laboratory reporting 34 ppb were cautioned to scrutinize their recoveries to bring them into a range closer to the mean. Interlaboratory precision for DDE and p,p'-DDT was excellent, and, considering the low concentrations present, the RSD values for dieldrin and o,p-DDT were acceptable. The sample was prepared by spiking serum used to prepare an earlier check sample (No. 35). Although the formulation values are reported as approximate, in-house analysis of the final formulation indicated that the correct values were as shown.

Table 2-7 shows the results reported by 16 laboratories for a fourth blood serum blind sample. The sample contained three actual residues and three spiked residues, one of which (PCP) was added only to enrich an already-present residue. Residue identity and quantitation were straight-forward because all peaks were resolved on the recommended GC columns. A formal laboratory performance ranking (Section 2H) was not prepared for this sample since only HCB and trans-nonachlor were spiked in known amounts to blank serum. Calculations were made, however, for review purposes based on the known values for HCB and trans-nonachlor and the average recovery as the true values for p,p'-DDE and p,p'-DDT. On this basis, good laboratory performance was demonstrated by participating laboratories with few exceptions. Of the 16 reporting laboratories, 9 would have scored above 190, 3 above 170, and 4 in the 116-147 range. The mean recovery for HCB was 99% and for trans-nonachlor 110%. The relative standard deviation (RSD) figures as shown in the accompanying table demonstrate good precision with the possible exception of p,p'-DDT. The 31% RSD for p,p'-DDT appears excessive at first glance but is certainly understandable considering that the low residue level of 2.6 ppb is close to the method sensitivity limit.

A 50 ppb spike was added to pooled serum containing PCP in an unknown amount. Three values were reported of 102, 142, and 190 ppb. A fourth value obtained by one laboratory was 180 ppb. The true value obtained by one laboratory was 180 ppb. The true value was probably in the 180-190 range, since analysis of the unspiked serum in the coordinating laboratory yielded 126 ppb. Compared to 180 ppb in the fortified sample, this gives a difference of 54 ppb, which is in excellent agreement with the actual spike of 50 ppb. The higher results obtained by the coordinating laboratory and laboratory No. 4 can be explained by the fact that a revised PCP method including a hydrolysis step to free conjugated residues was used by these laboratories but not by laboratories No. 7 and 25.

Although performance on sample 54 was generally good, significant quantitation error was observed in a few instances. The integrity of standard solutions was suspect in some results, but poor chromatography techniques were undoubtedly also major factors. Some specific examples based on careful analysis of results are: measuring small responses like 5-6 mm peak heights; injecting less than 3 µl; large differences in injection volumes of sample and standard; and large differences in peak heights of sample and standard. Careless mistakes were also evident. One laboratory spoiled an excellent set of data by identifying trans-nonachlor as heptachlor epoxide on the OV-17/OV-210 column. A re-injection on an OV-210 column was made, but identity was evidently not checked against heptachlor epoxide standard also on the same chart. This would have clearly signalled an identification problem. Two laboratories missed p,p'-DDT. The GC system did not appear sensitive enough in one case. However, from chromatograms submitted by the other laboratory, it appears that the sample chromatogram was not allowed to run sufficiently long to elute DDT. These kinds of problems are discussed in detail elsewhere in this Manual, especially in Section 5.

The results of 17 laboratories with a fifth serum blind sample are shown in Table 2-8. Although this sample represented a rather simple residue mixture, the necessity for a judicious choice of GC columns is well illustrated. Heptachlor epoxide and trans-nonachlor are well separated on the two mixed phases OV-17/OV-210 and SE-30/OV-210, but not on the single phase OV-210. Dieldrin and p,p'-DDE separate on SE-30/OV-210 or OV-210 columns, but an exceptionally efficient OV-17/OV-210 column would be necessary for accurate quantitation. All Office of Pesticide Programs project laboratories involved in the study correctly identified the six serum residues. Two non-project laboratories missed one compound each. Fifteen of the 17 participants had performance ratings in excess of 190 points (Table 2-18).

Table 2-9 gives results for a serum blind sample containing mirex. All participants except one correctly identified five pesticide residues, while only 3 laboratories identified mirex. The p,p'-DDE was not spiked but represented the actual residue in the pooled blood serum matrix. Mirex was not included in the scoring but was fortified at the 10 ppb level. Low recovery of mirex demonstrates the poor extraction efficiency of this compound in hexane. The results of laboratories as 24 and 52 and the coordinating laboratory indicate an extraction efficiency of ca 25%. This low level placed the mirex concentration below the GC sensitivity limit for several of the laboratories.

c. Fat Check Samples

Tables 2-10 through 2-12 show results for fat check samples. For sample 56 (Table 2-11), all reporting laboratories correctly identified the seven added pesticides. Dieldrin was not added as a spike, but was identified by 6 laboratories in amounts of 5-10 ppb.

Sample 70 (Table 2-12) was designed to measure the proficiency of a laboratory in recognizing and quantitating PCB contamination in an adipose sample containing common organochlorine pesticide residues. The pesticides and fortification levels were chosen based on data from national surveys so as to represent a realistic analytical problem. In the summary of results table, laboratories listed above the double line are Epidemiology/Human Monitoring Contract laboratories. This sample proved to be a very difficult challenge for the majority of the laboratories. The percentage total error (TE) figures demonstrate a generally unacceptable level of performance for this analysis. Five of the nine TE figures were greater than 50% and, therefore, "unacceptable." TE figures for only the EPA contract laboratories were: HCB, 51.3%; β-BHC, 16.4%; oxychlordane, 45.7%; trans-nonachlor, 22.3%; heptachlor epoxide, 43.5%; p,p'-DDE, 46.7%; p,p'-DDT, 30.1%; dieldrin, 42.7%; and PCB 1254, 120%.

d. Water Check Sample

Table 2-13 shows the results of an interlaboratory water round robin sample reported by 19 laboratories in 1977. The sample contained spikes of four parent organochlorine pesticides plus Aroclor 1254, but no partially altered compounds as would undoubtedly be present in routine monitoring samples. The results shown in Table 2-13 are acceptable considering the relative difficulty of the sample. Interlaboratory precision, as measured by the relative standard deviation values, are reasonable, and total error values, although above the 50% level considered satisfactory for less complex formulations, are not too far off on this particular sample. Mean recovery values for all laboratories, after rejecting outliers, were HCB, 67%, oxychlordane, 83%; p,p'-DDE, 85%; p,p'-DDT, 86%; and Aroclor 1254, 81%. The value for HCB is not as bad as indicated because the best recovery possible for HCB was 85% (0.25 ppb).

2H RELATIVE PERFORMANCE RANKING

a. Original Performance Ranking Scheme

A scheme has been used from the start of the QC program until 1980 for the relative ranking of laboratory results in the analysis of multiresidue check samples. This scheme, described in this section, was used to calculate the rankings shown in Tables 2-15 to 2-22. A new scoring procedure that has been adopted for future interlaboratory samples is described in Subsection 2Hb.

There are three essential criteria for a high score in the performance ranking, namely, correct identification of all pesticides present, correct quantitative assay of the pesticides, and non-reporting of pesticides not present. The ranking scheme incorporates all three criteria and provides a numerical score for each.

The maximum possible score is 200 points, 100 for correct identification and 100 for quantitation. A detailed explanation of the calculation procedure follows:

(1) Identification

The 100 possible total points divided by the number of compounds actually present yields the point value per compound. Correct identification of all compounds present and reporting of no extra compounds results in a total score of 100 points. A penalty equal to the point value per compound is assessed for each compound reported that is not actually present. For example, if five compounds are present in the check sample, each is worth 20 points. If one is missed and one extra is reported, a penalty of 2x20=40 points will be assessed against identification performance. The score in this part would then be 100-40=60 points.

(2) Quantitation

The point value per compound is again the total possible points (100) divided by the number of compounds present. Should all reported values coincide exactly with the formulation values (an unlikely situation), the full 100 points are awarded. When a reported value differs from the formulation value, the difference between the two (the absolute error) divided by the standard deviation (previously calculated for each compound) gives a "weighted deviation." This value is subtracted from the point value of the compound to give the quantitative score for that compound:

$$\text{Compound Quantitative Score} = \text{Compound Point Value} - \frac{\text{Absolute Error}}{\text{Standard Deviation}}$$

The total score for this part is the sum of the individual compound quantitative scores.

An important aspect of the quantitative portion of ranking is the role played by the standard deviation for each compound. If the precision of the group for the analysis of a particular pesticide is poor, the standard deviation for that compound will be relatively high. If a laboratory has a large absolute error for this one compound but an otherwise excellent performance, division of the error by the large standard deviation will lower the point loss so that an unduly heavy scoring penalty is not received.

(3) Total Score and Sample Results

The total score for laboratory performance is the sum of the identification and quantitation point totals. Table 2-14 illustrates in detail the method of calculation for a hypothetical analysis in which one compound is missed and one extra is reported, resulting in an inferior total score of 125.

Table 2-14

CALCULATION OF TOTAL SCORE FOR RELATIVE PERFORMANCE RANKING

	Formulation pg/µl	Reported Analytical Values pg/µl	Standard Deviation*
β-BHC	30	27	2.10
p,p'-DDE	40	40	1.75
Dieldrin	20	50	2.50
o,p'-DDT	10	Not Reported	0.60
p,p'-DDT	50	47	1.44
α-BHC	None	10	----

*Of all data from participating laboratories

Point value for each compound is 100 ÷ 5 = 20

Identification

β-BHC - 20

p,p'-DDE - 20

Dieldrin - 20

o,p-DDT - 0

p,p'-DDT - 20

sum = 80

-20 Penalty for false identification of α-BHC

60 Total identification points

Quantitation

β-BHC - $20 - \frac{30 - 27}{2.10} = 19$

p,p'-DDE - $20 - \frac{40 - 40}{1.75} = 20$

Dieldrin - $20 - \frac{20 - 50}{2.50} = 8$

o,p-DDT - - - - - = 0

p,p'-DDT - $20 - \frac{50 - 47}{1.44} = 18$

Total quantitation points = 65

Total laboratory score 60 + 65 = 125 (of a possible 200 points)

Tables 2-15 through 2-22 show Relative Performance Rankings for groups of laboratories on check samples of different types. Table 2-15 shows rankings for the laboratories reporting the data in Table 2-3. Laboratories with scores over 190 are considered to have demonstrated generally acceptable performance with some possible minor problems. Scores between 150 and 190 indicate definite problems that should be quickly resolved. Those with scores below 150 should suspend all routine pesticide analytical work pending the resolution of very serious problems in both identification and quantitation, the effects of which place in doubt all routine analytical data output of the laboratories. The laboratories are to initiate corrective action immediately based on the general and individual critiques received and personal consultations with the coordinating laboratory. The remaining portions of the original check sample can be used as a reference standard material to internally evaluate improvement before receipt of a new check sample to again test laboratory performance.

Each set of performance ranking data must be carefully appraised by highly skilled, experienced residue analysts in the coordinating laboratory before deciding upon what, if any, action should be taken based on the results. For example, Table 2-16 shows ranking data for 17 laboratories analyzing a fat sample (results reported in Table 2-10) and Table 2-17 a blood analysis performed by 16 laboratories. Examination of the scores for the fat sample indicates a significant breaking point between laboratories with 185 or more points and those with 168 or lower. Reference to Table 2-16 shows that those below the break point had readily apparent problems, and these four laboratories received corrective critiques. To the contrary, all rankings for the blood analysis were 192 or greater, and all laboratories were considered to have turned in acceptable performances, even those with the poorest relative scores.

Performance rankings for laboratories participating in the analysis of some later blind samples are shown in Tables 2-18 to 2-22. Rankings for blood serum sample No. 59 shown in Table 2-18 indicate that only two laboratories scored below 190; one laboratory scored below the 150 level indicating very serious problems requiring immediate resolution. Performance on serum sample No. 66 (Table 2-19) was also generally excellent, with all but one laboratory scoring above 190 points. Performance on fat sample No. 56 (Table 2-20) was also excellent, with 12 of the 16 laboratories scoring over 190 points and a low score as high as 175.9. Since there were no missed compounds, the scoring spread reflects entirely the ability of the laboratories to accurately quantitate residues. The lowest scoring laboratory did not use proper standards for some quantitations, so accuracy was understandably poor. The 12% recovery for heptachlor epoxide by laboratory 16 was also understandable because the analyst attempted to quantitate a peak height of only 8 mm against a 62.5 mm standard (see Section 50h). Fat sample No. 70 was an exceptionally difficult sample containing both common organochloride pesticides and PCBs. Only 5 laboratories achieved a score of 190 or above (Table 2-21). Performance on water sample No. 39 is shown in Table 2-22. Laboratories with scores above 170 had only minor problems, if any. Below this, there was sharp drop-off to a score of 115.9. The score of zero for the lowest laboratory, which was a new participant in the EPA AQC program, resulted because the sum of the penalty points exceeded the positive points.

Table 2-15

RELATIVE PERFORMANCE RANKINGS

CHECK SAMPLE NO. 26, MIXTURE IN SOLVENT

Lab. Code Number	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
161.	0	0	0	198
137.	0	0	0	198
135.	0	0	0	197
162.	0	0	0	197
87.	0	0	0	197
113A.	0	0	0	197
113.	0	0	0	196
85.	0	0	0	196
48.	0	0	0	195
130.	0	0	0	195
66.	0	0	0	195
73.	0	0	0	194
72.	0	0	0	194
84.	0	0	0	192
89.	0	0	0	192
88.	0	0	1	189
83.	0	0	0	189
96.	0	0	2	187
97.	0	1	0	181
164.	0	1	4	169
68.	1	0	1	168
92.	1	0	0	168
93.	0	1	2	164
90.	1	0	2	159
53.	1	0	1	158
163.	1	1	0	157
95.	2	0	0	146
160	2	1	0	133
45.	0	0	6	128
71.	3	0	3	127
52.	2	1	2	123
47.	3	0	1	115
69.	4	0	2	84
54.	4	4	4	25

1/ Values outside confidence limits
2/ Total possible score 200 points

Table 2-16

RELATIVE PERFORMANCE RANKINGS - CHECK SAMPLE NO. 21, FAT

Lab. Code Number	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
15.	0	0	0	198
16.	0	0	0	198
8.	0	0	0	198
25.	0	0	0	197
7.	0	0	0	195
4.	0	0	0	193
26.	0	0	0	191
33.	0	0	1	191
5.	0	0	0	191
34.	0	0	1	189
11.	0	0	1	188
9.	0	0	1	187
31.	0	0	0	185
6.	1	0	0	168
1.	1	0	0	168
14.	1	0	0	167
24.	0	1	3	165

1/ Values outside confidence limits

2/ Total possible score 200

Table 2-17

RELATIVE PERFORMANCE RANKINGS - CHECK SAMPLE NO. 23, SERUM

Lab. Code Number	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
1.	0	0	0	198
2.	0	0	0	198
3.	0	0	0	197
4.	0	0	0	197
5.	0	0	0	197
6.	0	0	0	197
7.	0	0	0	196
8.	0	0	0	196
9.	0	0	0	196
10.	0	0	0	196
11.	0	0	0	196
12.	0	0	0	195
13.	0	0	0	194
14.	0	0	0	193
15.	0	0	1	192
16.	0	0	1	192

1/ Values outside confidence limits

2/ Total possible score 200

Table 2-18

RELATIVE PERFORMANCE RANKINGS

CHECK SAMPLE NO. 59, BLOOD SERUM

Lab Code No.	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
8	0	0	0	198.04
15	0	0	0	197.72
26	0	0	0	197.31
25	0	0	0	197.08
4	0	0	0	196.54
11	0	0	0	196.51
14	0	0	0	195.90
5	0	0	0	195.41
1	0	0	0	195.30
13	0	0	0	194.37
7	0	0	0	194.05
16	0	0	0	193.49
6	0	0	0	193.30
32	0	0	0	191.60
12	0	0	1	191.52
9	1	0	0	162.41
34	1	0	4	147.15

1/ Rejected as outliers

2/ Total possible score - 200 points

Table 2-19

RELATIVE PERFORMANCE RANKINGS
CHECK SAMPLE NO. 66, BLOOD SERUM

Lab. Code No.	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
52	0	0	0	199.3
25	0	0	0	197.7
12	0	0	0	197.6
4	0	0	0	196.9
8	0	0	0	196.7
24	0	0	0	195.7
38	0	0	0	195.5
26	0	0	0	195.5
7	0	0	0	195.4
14	0	0	0	194.1
1	0	0	0	194.0
13	0	0	0	193.8
5	0	0	1	192.0
6	0	0	0	191.0
16	0	0	0	190.8
11	1	0	0	153.1

1/ Rejected as outliers

2/ Total possible score - 200 points

Table 2-20

RELATIVE PERFORMANCE RANKINGS
CHECK SAMPLE NO. 56, FAT

Lab. Code No.	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
15	0	0	0	197.60
38	0	0	0	196.29
1	0	0	0	195.42
25	0	0	0	194.87
11	0	0	0	193.67
8	0	0	0	193.17
12	0	0	0	192.59
4	0	0	0	192.57
6	0	0	1	192.19
14	0	0	0	191.89
34	0	0	0	191.63
7	0	0	0	190.54
13	0	0	0	190.29
26	0	0	1	189.09
16	0	0	2	184.69
31	0	0	2	175.87

1/ Rejected as outliers

2/ Total possible score - 200 points

Table 2-21

RELATIVE PERFORMANCE RANKINGS
CHECK SAMPLE NO. 70, FAT

Lab. Code No.	Compounds Missed	False Identifications	Identification Score	Quantitation Score	Total Score
51*	0	0	100	95.57	195.57
8*	0	0	100	92.89	192.89
26*	0	0	100	91.17	191.17
38	0	0	100	90.37	190.37
4*	0	0	100	90.24	190.24
52	0	0	100	73.80	173.80
25*	0	2	77.78	94.03	171.81
11*	1	0	88.89	81.30	170.19
7*	0	1	88.89	80.99	169.88
16	1	0	88.89	79.14	168.03
14*	1	0	88.89	77.68	166.57
13	1	0	88.89	77.12	166.01
9	1	0	88.89	75.95	164.84
12*	1	1	77.78	80.62	158.40
24*	1	1	77.78	78.38	156.16
6	2	0	77.78	73.33	151.11
10*	1	2	66.67	83.21	149.88
1	2	3	44.44	69.10	113.54

* Epidemiology/Human Monitoring Contract Laboratory

Table 2-22

RELATIVE PERFORMANCE RANKINGS
CHECK SAMPLE NO. 49, WATER

Lab. Code No.	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
5	--	--	--	195.72
3	--	--	--	195.37
25	--	--	--	195.14
15	--	--	--	194.31
8	--	--	--	191.87
11	--	--	--	188.93
34	--	--	1	183.37
36	--	1	1	171.40
13	2	--	--	115.92
26	2*	--	--	115.49
7	2	--	--	114.42
10	2	1	--	94.0
16	1	--	4	80.0
1	3	--	--	78.75
9	1	4	1	74.22
24	3	--	1	73.74
12	3	1	--	56.57
6	4	--	--	39.14
23	3	4	1	0.00

* Later reported the presence of the two compounds

1/ Rejected as outliers

2/ Total possible score - 200 points

b. Current Performance Ranking Scheme

The new scheme adopted by the coordinating laboratory for ranking laboratory performance on interlaboratory check samples differs from the original only in the scoring of the quantitative results. The purpose of the change is to cause a greater point loss for laboratories with significant quantitation errors. This, in turn, will improve the relative performance of laboratories with more accurate results.

The new procedure involves dividing the absolute error by the standard deviation (SD) to obtain the "weighted deviation" as before (Subsection 2Ha). The score for each compound is obtained by squaring the weighted deviation and subtracting from the compound point value.

Weighted deviation	Point loss
0-1 standard deviations	0-1
1-2	1-4
2-3	4-9
3-4	9-16
4-5	16-25

The scoring penalty cannot exceed the point value per compound. It is felt that this approach more fairly penalizes large errors but is not overly harsh for results with small errors.

As a specific example, the quantitative scoring shown in Table 2-14, would change in the following manner under the new scoring system.

Compound	"Weighted deviation"	Points Subtracted	Score
β-BHC	$\frac{30-27}{2.10} = 1.4$	1.96	20-1.96 = 18.0
p,p'-DDE	$\frac{0}{1.75} = 0$	0	20-0 = 20
Dieldrin	$\frac{30}{2.50} = 12$	20	20-20 = 0
o,p'-DDT	not reported	—	20-20 = 0
p,p'-DDT	$\frac{3}{1.44} = 2.1$	4.41	20-4.41 = 15.6
		Total quantitation points =	53.6

The major difference is the loss of all quantitation points for dieldrin, which certainly seems fair considering the deviation of 12 units. As before, all points are lost for compounds not correctly identified (o,p'-DDT).

Experience will have to be accumulated using the new scoring method in order to assess how the numerical values for satisfactory performance will vary compared to the scores calculated with the old formula.

2I PRIVATE CRITIQUES

As already mentioned, laboratories with significant analytical problems receive added assistance in the form of a private, individual critique of their results reported for a check sample. The content of this critique depends upon the problems that are obvious from a careful analysis of the submitted results and might include comments on incorrect standards, instrumental factors, calculation errors, poor choice of materials or parameters, etc.

2J PROGRESSION OF PERFORMANCE

During the early years of the Interlaboratory Quality Control Program, results were expectedly poor. Methodology, equipment, and reagents were a matter of individual laboratory preference, and the first priority was development of uniform methodology and standardization among all laboratories.

As an example of the improvement attainable from such a program, the recovery and precision results on interlaboratory fat check samples for a group of human monitoring laboratories over a period of years are shown in Table 2-23. The method used in the first year was based on gas chromatography of a concentrated tissue extract without cleanup (1). Although the method was fairly rapid and simple, it was discovered that the GC column and detector became rapidly contaminated by repeated injection of uncleaned samples, and the check sample results proved the method was unsuitable for routine use by a laboratory network. Not only was precision poor as measured by the RSD, but the spread from minimum to maximum recoveries for several compounds was extremely wide, and mean recoveries were generally far from correct.

Conversion was made to a procedure including cleanup of the extract by acetonitrile/petroleum ether partition and Florisil column chromatography (2), resulting in significant improvement in not only precision (sample 9, Table 2-23) but in accuracy as well. After several months' experience with the method, results on another check sample (sample 11, Table 2-23) were even better, and with continued participation in the program, the laboratories made still further progress in their performance through 1974 as the figures in the Table show. The 1978 fat check sample results indicate an apparent reversion to 1972 precision levels. Since methodology has not changed, the results apparently reflect need for reestablishment of a training program for pesticide residue chemists such as was once conducted by the EPA Perrine Primate Laboratory in Florida. The 1979 results are also indicative of training needs plus the significant complications of analyzing organochlorine pesticide residues in the presence of PCBs.

The results in Table 2-24 show progression of laboratory performance on interlaboratory blood check samples between 1968 and 1979. In the beginning the

Table 2-23

PROGRESSION OF RESULTS
FAT CHECK SAMPLE

Interlaboratory Sample Number	Year	No. of Labs	No. of Compounds	Average Recoveries, %	Average RSD, %
3	1967	15	7	- 2/	50
9	1968	21	7	- 2/	38
11	1969	19	7	108	24
21 1/	1972	16	7	89	19
24	1973	14	7	95	14
28	1974	10	7	96	12
56	1978	16	7	92	19
70	1979	18	9 3/	91	23

1/ Complete data given in Table 2-10

2/ Unspiked samples for which exact pesticide levels were unknown

3/ Sample contained eight single component compound plus Aroclor 1254

Table 2-24

PROGRESSION OF RESULTS
BLOOD CHECK SAMPLE

Interlaboratory Sample Number	Year	No. of Labs	No. of Compounds	Average Recoveries, %	Average RSD, %
6	1968	22	6	*	36
10	1969	20	5	*	29
16	1970	22	4	*	21
17	1971	20	4	*	17
22	1972	17	4	96	14
23	1972	17	4	91	12
25	1973	18	4	100	13
27	1974	15	3	*	16
31	1975	17	4	*	20
33	1975	13	4	*	13
40	1976	14	4	*	17
46	1976	14	4	*	18
54	1977	16	4	*	18
59	1979	17	6	*	20
66	1979	16	5	*	15

* Unspiked samples for which the actual pesticide levels were not known

direct hexane extraction method of Dale et al. (3) was adopted but was found to yield poor interlaboratory precision. Sample 10 was analyzed by a triple extraction modification of this procedure, which also proved inadequate. The later samples were done with the currently recommended Thompson and Walker (4) extraction method, which utilizes a constant speed mixer (Subsection 9D). The results of the blood check samples illustrate again the dual value of the Interlaboratory Control Program in upgrading laboratory performance and in identifying weak analytical methodology.

2K STATISTICAL TERMS AND CALCULATIONS

a. Accuracy and Precision

Precision refers to the agreement or reproducibility of a set of replicate results among themselves without assumption of any prior information as to the true result. Precision is usually expressed in terms of the deviation, variance, or range. Accuracy is the nearness of a result or the mean of a set of results to the true value. Accuracy is usually expressed in terms of error, bias, or percentage recovery.

Good precision often is an indication of good accuracy, but one can obtain good precision with poor accuracy if a systematic (determinate) error is present in the method used. Systematic errors are either positive or negative in sign.

The other general classification of errors in analysis is indeterminate (random) errors. These are errors inherent in the analytical methods because of uncertainties in measurements. An example is the measurement of the height and width of a gas chromatographic peak with a ruler, which requires estimation between the mm division lines. Indeterminate errors are random, that is, they are just as likely to be positive as negative. For this reason, the average of several replicate measurements is always more reliable than any of the individual measurements. Although random errors are unavoidable, determinate errors can be corrected once their cause is located.

Standards of accuracy and precision are not the same for a residue analysis as for a macro analytical method such as a titration, for which a precision and accuracy of 1-5 parts per thousand is usually expected of an experienced analyst. The analysis of technical pesticide products is also a macro method for which accuracy and precision are fundamental factors, and the measurement step (usually internal standard GC or LC) must be carried out with this in mind. In contrast with macro methodology, residue analysis involves the assay of nanogram or lower amounts of pesticides, and with the extensive cleanup and great amount of experimental manipulation required, procedures are considered adequately quantitative when values ± 15-20% or better are obtained on recovery samples fortified at ppm levels, ± 30% at ppb levels. One authority has suggested that a relative standard deviation or coefficient of variation (Section 2Ke) of less than 40% is acceptable for precision between laboratories for a trace analytical method. A model has been presented (5) to analyze the reproducibility of results of determinations of unknown amounts of pesticides in relatively few samples. The reliability of the analytical procedure, the influence of sampling techniques, and the number of samples that should be analyzed can be determined with the model.

Absolute error is the difference between the experimental result and the true value. Relative error is absolute error divided by the true value and multiplied x 100 to yield percent relative error or x 1000 to yield parts per thousand relative error. As an example, an absolute 0.2 µl error in injection of a sample for GC corresponds to $\frac{0.2 \times 100}{1.0} = 20\%$ for a 1.0 µl sample but only $\frac{0.2 \times 100}{100} = 4\%$ for a 5.0 µl sample. It is explained later in Section 5 that low sample injection volumes are to be avoided because of high potential errors. Bias is defined as the mean of the differences (having regard for signs) of the results from the true value.

b. Significant Figures

The uncertainty of a piece of data is assumed to lie in the last digit recorded, and unless qualifying information is given this last digit is assumed uncertain by ± 1. If the height of a GC peak is reported as 10.0 cm, the absolute uncertainty is ± 0.1 cm, and the relative uncertainty is $\frac{0.1}{10} \times 100 = 1\%$. Likewise, one should always be sure to record all certain figures plus one uncertain figure in a measurement, these figures being designated as significant figures.

Only significant figures should be used in recording and calculating analytical results. If the value 12.3 mg/g is reported for a pesticide analysis, the 12 should be certain while the 3 is more or less uncertain. Good judgment on the part of the analyst is required to decide on the proper number of figures so that significant digits are not lost or non-significant ones retained. All numbers written after the first real number are considered significant. The numbers 1.23, 12.3, and 123 all have three significant figures. Zeroes can cause some problems and should be paid special attention. Zeroes written before the first real number are not significant but merely serve to locate the decimal place. Therefore, the numbers 0.123, 0.0123, and 0.00123 all have three significant figures; the number 0.1012 has four significant figures since the second zero follows the first real number (in this case 1) and is, therefore, significant. All terminal zeroes following a decimal point are significant. For example, 9.800 g indicates a weight of 9.8 grams accurate to the nearest 1 mg. All four figures are significant. The number 10,100 should indicate five significant figures, but terminal zeroes in a whole number must be considered with suspicion because the proper rule is not carefully followed. If the value 10,100 mm indicates that measurement was made to the nearest 1 mm, the absolute uncertainly is ± 1 mm and the relative uncertainty $\frac{1}{10{,}100} \times 100 = 0.01\%$. If the measurement was actually made to the nearest 0.1 meter and the final zeroes only indicate the magnitude of the number in mm, the number would better be written in exponential form, 1.01×10^4 mm, to indicate an absolute uncertainty of $\pm 1 \times 10^2$ mm and a relative uncertainty of

$$\frac{1 \times 10^2}{10{,}100} \times 100 = 1\%.$$

Significant figures should be properly retained when performing mathematical operations. Simplified rules that serve in most cases are as follows. In addition or subtraction, the answer has as many decimal places as the number with the fewest decimal places. For example:

$$\begin{array}{rcr} 90.7 & \text{----------} & 90.7 \\ 8.81 & \text{----------} & 8.8 \\ +\ 0.551 & \text{----------} & 0.6 \\ \hline & & 100.1 \end{array}$$

Inspection of the three numbers to be added indicates the answer can have only one decimal place. Each number is initially rounded off to one decimal place and then the sum is taken. Note that the correct answer has four significant figures (even though each number added had only three) but only one decimal place. Rounding off is done by rounding the last retained digit up if the discarded digit is greater than or equal to 5; the last digit is retained unchanged if the discarded digit is less than 5. For multiplication and division, the answer can have no more significant figures than the number with the fewest significant figures. For example, in calculating the ng of pesticide represented by an unknown GC peak by comparison with the area of a standard peak, the formula $ng_u = ng_s \frac{area_u}{area_s}$ is used if response is linear over the range in question. If 1.0 ng standard gives a peak of 9.0 cm height (measured to the nearest 1 mm) and the unknown peak height is 12.0 cm, the ng of unknown is $1.00 \times \frac{12.0}{9.0} = 1.3$ ng with only two significant figures reportable. If an analysis is based on peak areas calculated by the usual formula height x width at one-half height, a width of less than 10 cm measured only to the nearest one mm limits the area and the calculated amount of pesticide to two significant figures.

c. Average

The average or mean ($\overline{X}$) of a set of n values is calculated by summing the individual values and dividing by n:

$$\overline{X} = \frac{\Sigma\, Xi}{n}$$

d. Range

The difference between the highest and lowest values in a group.

e. Standard Deviation and Variation

Standard deviation(s) of a sample of n results is calculated by use of the equation:

$$s = \left[\frac{\Sigma\, Xi^2 - \frac{(\Sigma\, Xi)^2}{n}}{n-1} \right]^{1/2}$$

Variance is equal to s^2. Relative standard deviation (RSD) or coefficient of variation (CV) is the standard deviation divided by the mean and multiplied by 100 (percentage) or 1000 (parts per thousand). σ is the standard deviation for a very large set of data, calculated by the above equation with n rather than n-1 in the denominator. Precision is increased (value of s reduced) by increasing the number of replicate analyses, enabling one to determine with greater statistical confidence that the true mean lies within certain limits about the experimental mean or to reduce the interval at a certain confidence level. Confidence limit or interval is defined as:

$$\mu = \overline{X} \pm \frac{ts}{\sqrt{n}}$$

where μ is the true mean, $\overline{X}$ is the experimental mean, and t is a value obtainable in tables for different percentages of confidence and numbers of trials (n). Values of t increase as percentage confidence desired increases and decreases as the number of replicates increases.

f. Fitness Test

EPA Quality Assurance personnel have applied the following test for rejection of "outlier" values in check sample data, which, if left in, would exert a significant effect on the overall data:

(1) Compute the mean and the standard deviation of the entire data set.

(2) Compute the absolute value of the arithmetic deviation from the mean of all values in the data set.

(3) Establish the correct factor to be used in the calculation (Step 4) from the following table.

Number of data points (n) in the data set	Factor
5	1.65
6	1.73
7	1.81
8	1.86
9	1.91
10	1.96
12	2.04
14	2.10
16	2.15
18	2.20
20	2.24
25	2.33
30	2.39
40	2.49

(4) If the absolute value of the arithmetic deviation from the mean for any number in the data set is greater than the factor from step (3) times the standard deviation of the entire data set, the number is rejected as lying outside a reasonable data set.

(5) The percent confidence interval for the retained values would be given by:

$$\left(1 - \frac{1}{2n}\right) 100 = \%$$

This Fitness Test has proven to be practical and reasonable over many years with round robin interlaboratory blind sample exercises wherein proven methodology is used. It is based in part on Chauvenet's criterion as described by Hugh D. Young (6). Individual statisticians disagree on the best test for rejection of questionable results, and no claim is made for the rigorous statistical validity of the method described in this subsection.

g. Total Error

Total error is a method proposed by McFarren et al. (7) for combining precision and accuracy in one reporting expression:

$$\text{Total Error} = \frac{\text{Absolute Value of the Mean Error} + 2s}{\text{True Value}} \times 100$$

where s = standard deviation. In general, total error values $\leq$ 25% are considered excellent, $\leq$ 50% acceptable, and > 50% unacceptable.

Specifically, in the interlaboratory control program, total error is calculated from the following equation:

$$\text{Total Error} = \frac{x + 2s}{y}$$

where x = the arithmetic deviation of the overall mean obtained for a given pesticide from its known formulation value (the absolute value of the mean error), y = the formulation (true) value, and s = the standard deviation. A discussion of this equation has recently been published (8), indicating it may unnecessarily downgrade a considerable portion of results. Alternative equations are recommended which rigorously meet the McFarren et al. 25 or 50% criterion with at least 95% confidence. These equations are:

$$T = \frac{x + 1.7\ s}{y} \times 100$$

to be used when $x/s > 0.3$ and up to 44 results are available,

$$T = \frac{x + 1.8\ s}{y} \times 100$$

when $x/s = 0.3 - 0.15$ and number of results are 45-170, and

$$T = \frac{2\ s}{y} \times 100$$

when x is not significantly different from zero with 95% confidence.

h. Numerical Conversions

$1\ \text{g} = 1000\ \text{mg}$

$1\ \text{mg} = 1000\ \mu\text{g} = 10^{-3}\ \text{g}$

$1\ \mu\text{g} = 1000\ \text{ng} = 10^{-6}\ \text{g}$

$1\ \text{ng} = 1000\ \text{pg} = 10^{-9}$

$1\ \text{pg} = 10^{-12}\ \text{g}$

$1\ \text{ml} = 1000\ \mu\text{l} = 10^{-3}\ \text{liter}$

$1\ \mu\text{l} = 10^{-6}\ \text{liter}$

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2M ADDITIONAL SOURCES OF INFORMATION ON PESTICIDE QUALITY ASSURANCE PROGRAMS

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Section 3

INTRALABORATORY QUALITY CONTROL

3A PURPOSE AND OBJECTIVES

The intralaboratory control program is a continuing, systematic, inhouse regimen intended to ensure the production of analytical data of continuing high validity. Several of the program objectives are parallel to those given in Section 2 for the interlaboratory program:

a. To provide a measure of the precision of analytical methods.

b. To maintain a continuing assessment of the accuracy and precision of analysts within the laboratory group.

c. To identify weak methodology and provide a continuing source of research problems aimed at overcoming deficiencies.

d. To detect training needs within the analytical group.

e. To provide a permanent record of instrument performance as a basis for validating data and projecting repair or replacement needs.

f. To upgrade the overall quality of laboratory performance.

The following subsections will treat several integral parts of a high quality intralaboratory quality control program, embracing such areas as the periodic analysis and interpretation of results of spiked reference materials (SPRM's), instrumental maintenance and calibration, and monitoring of the quality of various materials used in the analytical scheme.

3B PURPOSE AND OBJECTIVES OF SPRM'S

In contrast to the interlaboratory check sample program in which one analyst in a laboratory will analyze a sample occasionally sent by the coordinator, the intralaboratory SPRM program provides a continuing measurement of the performance capability of each analyst. Each person can be constantly aware of his strengths and weaknesses, and corrective steps can be undertaken when necessary, before serious problems occur and erroneous data are reported out of the laboratory.

The program involves continual, systematic recovery studies on prepared test samples of each type of substrate routinely analyzed by a laboratory. Each staff chemist conducting routine analyses should participate, and all recovery results are recorded on a table available for examination by the chemist's supervisors.

3C NATURE OF SPRM'S

One possible approach is for a laboratory to prepare its own SPRM's. If the laboratory routinely analyzes animal fat samples, an appropriate check sample may be prepared as follows: Obtain a local bulk sample of 2 lb. or more of fatty tissue, place in a large beaker, and warm carefully on a hot water bath to a temperature not above 50°C with intermittent stirring. After a sufficient quantity of liquid fat has been expressed, filter into a second beaker through glass wool (pre-extracted with hexane) held in a glass or porcelain funnel. Heat the filtered fat to ca 45°C, transfer about one-half to a previously tared flask with standard taper stopper, and reweigh to the nearest 0.1 g. This portion is stored in the stoppered flask in a freezer at -18° to -23°C for later spiking. The remaining half is divided into individual analysis units in small vials or bottles that are also stored in the freezer. The weight of each unit is slightly larger than the intended sample weight. These serve as unspiked SPRM's.

Sufficient analyses are made on the unspiked subsamples so as to be satisfied with the reproducibility of results from the same analyst and among all participating analysts. For verification, the sample may be sent to an outside laboratory with experience in performing the analysis in question. When reproducibility is sufficient to establish a reliable pesticide profile in the unspiked sample, the other half is spiked to produce residue levels approximating or slightly exceeding the levels obtained in routine media. The spiked fat is thoroughly mixed, transferred to small bottles, and stored in a freezer. These spiked samples serve to test the capability of the analyst for recovery of higher pesticide levels.

For both the unspiked and spiked SPRM's, at least a dozen replications of the analysis on the same sample should be conducted by chemists with recognized competence. From these data, the percentage relative standard deviation is calculated and used in construction of control curves as described later in Subsection 3F.

The same basic program outlined for fatty tissue can be followed for other sample materials. If the compound(s) and media are known to be fully stable at room or refrigerator temperature, freezer storage is not required.

The EPA-ETD Interlaboratory Program provides participating laboratories with a sufficient supply of each interlaboratory check sample to serve also as an intralaboratory SPRM for a six month period (Subsection 2D).

Laboratories should store the excess material in sample-size portions in a freezer to be withdrawn periodically for analysis along with routine samples. The correct formulation value will be known to the laboratory supervisor when he receives the interlaboratory Summary of Results Table (Subsection 2G) from the coordinator, so that he can compare the results of his personnel with the "correct" values. The advantage of this second approach is that a participating laboratory will have internal SPRM's with reliable results available to them without having to prepare their own samples and establish residue levels and RSD values before they can be routinely used.

Because of their nature, it has not been the practice to treat intralaboratory SPRM's as blinds in the EPA program. A homogeneous, frozen fat check sample in a vial, which is simply dissolved in hexane as the first analytical step, would be difficult to camouflage as a routine fat sample, normally encountered by the chemist as a chunk of adipose tissue requiring initial grinding (EPA Pesticide Analytical Manual, Section 5,A,(1),(a),III,3). Likewise, routine blood samples are received as a whole blood rather than as the serum form of the check sample. It would be undoubtedly advantageous to devise SPRM's that could be offered to the chemist as a true blind along with his normal sample load, but this has proven a difficult task with fat and blood when it is necessary to prepare a homogeneous sample guaranteed to give a consistent analysis regardless of the portion taken. It might well be feasible for some other sample substrate, such as urine or water.

3D FREQUENCY OF SPRM ANALYSIS

The frequency of SPRM analysis is related to the volume of routine samples run. Laboratories making less than one routine analysis per week of a given substrate should analyze a corresponding SPRM sample with each routine sample, and not less than one SPRM analysis per month even if no routine samples are encountered. Laboratories analyzing one or more samples per week should analyze at least 10 percent as many SPRM samples as routine samples, with a minimum of one per week. For example, if one to fourteen samples are run per week, at least one standard sample should be analyzed each week. If thirty samples are run, one corresponding SPRM sample should be analyzed for each nine samples, or a total of three standard samples. The SPRM is carried through the analysis in parallel with a group of routine samples, giving it no special care or treatment.

In laboratories where more than one chemist performs an entire routine analysis of a given substrate, each individual should run separate SPRM samples. However, if protocol is that routine analyses are handled by a team, e.g., with one chemist preparing extracts and another doing the determination, SPRM samples should be handled in this same normal fashion.

3E RECORD KEEPING

Immediately upon completion of each analysis of an SPRM sample, results are recorded on an Internal Check Sample Form. An example for blood serum is shown as Table 3-1. Data are entered in legible handwriting. Each participating chemist should have access to this record. If significant deviations from the furnished correct (mean) values occur, an investigation is begun at once to determine the reason or reasons.

The chief chemist of each laboratory completes a quarterly report for forwarding to the coordinator and includes in the confidential in-house section (Table 3-2) one copy of each Internal SPRM Report. The coordinator compiles the data from all laboratories and furnishes to each statistical summaries for comparison of results.

The following two publications by the National Enforcement Investigations Center (Denver Federal Center, Bldg. 53, Box 25227, Denver, CO 80225) of the EPA contain information on record keeping and reporting of analytical results:

(a) NEIC Policies and Procedures Manual, May, 1978.

(b) Pesticide Product Laboratory Procedures Manual, August, 1979.

Reference (a) outlines legally oriented standard operating procedures for EPA chemistry and biology laboratories. Pages II-19 to II-29 focus on document control, with information on serialized documents, project logbooks, field data records, sample identification documents, chain-of-custody records, analyst and instrument logbooks, photographs, document corrections, document consistency, document numbering and inventory, and files.

The information in Reference (b) is specifically for laboratories performing analytical testing on pesticide formulations and products to determine if labeling is correct. The results of these analyses can lead to a number of legal actions, including criminal action. Although it may be more important that records be kept carefully and completely in this situation compared to a monitoring laboratory, the same principles can be applied to all analytical laboratories. Analysts are specifically referred to Section IV of Reference (b) above for recommended procedures on two aspects of sample custody: documentation and physical sample security. These procedures are designed to ensure that collected samples are not tampered with in the event of any subsequent legal action.

Section VI of Reference (b) describes proper record keeping. Although this material is not uniquely applicable to SPRM samples, the points of importance to all pesticide analysts will be summarized here. The reader is also referred to Section 50d of this Manual for further information on reporting of results and record keeping.

TABLE 3-1

RECORD OF ANALYSIS OF STANDARD REFERENCE MATERIAL

Laboratory__________________

____
Media

Analyst or Team___________________

Sample No.	Date	Aldrin	β-BHC	Hept. Epox.	Dieldrin	o,p'-DDT	p,p'-DDD	p,p'-DDE	p,p'-DDT	

Analyst or Team______________________

Reporting units should be in ppb or ppm. Observe standing instructions for minimum reporting levels.

TABLE 3-2

____
(Date)

SUBJECT: Quarterly Report, Quarter Ending ____________________

TO: Chief, Quality Assurance Section, Analytical Chem. Branch, Health Effects Research Laboratory, (MD-69), Research Triangle Park, NC 27711

FROM: Chief Chemist ____________________(Laboratory)

During the past quarter we have analyzed the following numbers of routine* samples for pesticide residues:

Blood (multiresidue)	______	
Blood (PCP)	______	
Blood (Other)	______	(specify)
Adipose Tissues	______	
Other Human Tissues	______	
Air	______	
Soils	______	
Stream Sediment	______	
Water (multiresidue)	______	
Water (Other)	______	(specify)
Urine (alkyl phosphate)	______	
Urine (Other)	______	(specify)
Housedust	______	
Fish or Shellfish	______	
Wildlife	______	
Other**	______	

Are any spiked SPRM's prepared in-house? Yes No If Yes, list the substrates on the reverse side of this sheet giving the spiking level range of each compound spike.

____
Chief Chemist

*The term "routine" is intended to mean samples of local origin such as donors, autopsies, etc.

**Specify substrates if 10 or more samples were analyzed during quarter.

Detailed and specific notes should be made regarding all sample analyses, manipulations, and observations. Sufficient detail should be provided to enable the analyst or others to reconstruct the analysis step-by-step at a later date. All analytical work (graphs, charts, notes, etc.) should be retained in a general laboratory locked file cabinet and identified by sample number. This is in addition to the individual analyst's notebook or logbook and will assure that all primary information regarding a sample is in one location so that there is less chance of loss. Laboratory notebooks should be the "two-page" carbon or pressure-sensitive paper type. The originals are then removed from the notebook and retained with the laboratory records.

Careful notes should be made concerning the sample as received (see Subsection 8D), the preparation of the sample for analysis, and the actual determination. If a specified procedure is being followed, this should be referenced, and any variations from the procedure must be recorded. If the method is not specified, details of every step are recorded. Each laboratory operation should be accurately documented as to date performed, particularly when an analysis or several analyses of a sample or samples extend beyond one day. Time of starting and stopping should be recorded for all operations when duration is a factor, e.g., extractions, separations, centrifugations, color formations, etc.

Photographs can be made when they might be useful, e.g., to record the results of a thin layer chromatographic separation. All photocopies should be mounted on heavy paper and identified as to sample number, date, analyst, and subject matter.

Custody information and storage location should be documented if samples are stored overnight.

Reference standard information, including source, purity, and age should be recorded along with appropriate weighing and dilution data. If a reference standard is used that was prepared at an earlier date, then the original weighing and dilution data should be referenced.

All instrumental conditions should be recorded either on the worksheet or on an appropriate chart, graph, or printout. All graphs, charts, and printouts should be identified by sample number, date, analyst, and determination number.

Gas chromatography data should be recorded for each analysis at least to the following extent:

1. Gas chromatograph	- Make, model, and detector. Include designation if more than one of same model is available.
2. Column	- Source and/or date prepared - Length, id, od, and composition - Packing (%, type, and source)

3. Conditions	- Temperature of oven, injection port, detector, transfer lines, etc. - Flow rates, composition and purity of carrier, detector, and purge gases - Electrometer conditions such as range, attenuation, voltage, amperage, etc.
4. Injection	- Amount injected and size of syringe
5. Response	- Digital integration (incl. make, model, slope sensitivity, and other pertinent parameters), planimeter, peak height, cut and weigh, etc.
6. Internal standard (if used)	- Identification, source, and concentration
7. Any conditioning or calibration	
8. Recorder	- Make, model, range, and speed
9. Sensitivity	- % response to pg, ng of a standard material

HPLC data to be retained for each analysis should include at least the following:

1. Liquid chromatograph	- Make, model, type, and lab designation
2. Detector	- Make, model, type, and wavelength
3. Column	- Source and/or date prepared - Length, id, od, and composition - Packing (type, source, and particle size) - Pre-column, if applicable
4. Mobile phase	- Isocratic or gradient? - Name and % of each solvent - Degassed? Filtered?
5. Injector	- Type, make, and model - Amount injected

6. Temperature - Type of control and temperature

7. Sample handling - Filtration? Pore-size of filter

8. Response measurement - Digital integration (incl. make, model, and settings), planimeter, peak height, etc.

9. Recorder - Make, model, range, and speed

10. Internal standard - Identification, source, and concentration

Spectrophotometric data should be retained to the extent called for on the specific charts, along with any additional information as may be relevant to the measurement.

3F QUALITY CONTROL CHARTS

In addition to recording numerical results of each analysis of an internal SPRM, it may be desirable for each analyst or team to construct a Quality Control Chart. This depends to a great extent on the number of SPRM analyses of a given substrate per week or month. The purpose of this chart is to provide graphic assessment of accuracy and precision for the analysis of each substrate and instant detection of erroneous data. The charts allow quick and easy observation of recovery trends for a particular analysis and have long term value for the self evaluation of analytical output by staff personnel. Another significant value of the charts is that of providing a laboratory administrator with a rapid assessment of the continuing analytical capability of the staff chemists as related to the output of valid analytical data.

The first and very important step in the development of a control chart is the determination of an appropriate value of the relative standard deviation (RSD) (Subsection 2Ke) for the particular analysis. The RSD value used in preparation of control curves should be determined as suggested below, and should be a fixed value that represents the best precision possible for this particular method and substrate. This value, when once established, should then remain fixed for an indefinite period of time or until a method revision or improvement is made that would permit the determination of a lower RSD. A separate RSD value could be calculated and used for each pesticide residue in each method-substrate combination. However, this is unnecessarily complicated for a multi-residue method. A preferred practice is to determine an RSD value for several pesticides analyzed by a given method, and the average RSD value which will remain fixed as previously mentioned. An example would be the 10% RSD figure that is commonly accepted for all organochlorine residues determined by the EPA PAM procedures for blood serum or adipose tissue.

An in-house RSD value should be determined as suggested in Section 3C of this Manual: "at least a dozen replications of the analysis on the same sample should be conducted by chemists with recognized competence. From these data, the percentage relative standard deviation is calculated and used in construction of control curves." As a satisfactory alternative, two or more competent chemists of the staff analyze six replications of the same sample, from which a reasonable value for percentage RSD is obtained.

In summary, the RSD value is a measure of the best possible precision obtainable with a method. The accuracy of the method is, of course, not reflected in this figure. The quality control charts, however, provide a rapid assessment of both accuracy and precision.

The preparation of QC charts is illustrated by the following Figures 3-A and 3-B in which results for serum intralaboratory SPRM analyzed over a period of three months for p,p'-DDE and p,p'-DDT by chemists in two different laboratories are shown. (Several additional pesticides were also found, but only two are illustrated). Consecutive results are plotted on every second space along the X-axis. The Y-axis contains zero (0), plus (+), and minus (-) lines. The (+) line represents two standard error units (comparable to standard deviations) on the high side from the "correct" answer (the spiking level, or the level found by an experienced analyst in the coordinating laboratory), while the (-) line represents two standard error units (SEU) on the low side. In the case of this sample, it had been previously determined that an appropriate RSD value was 10% of the spiking level for each pesticide.

The known formulation or spiking value is subtracted from the experimental value (obtained for an analysis of the in-house standard sample to provide a (+) or (-) arithmetic deviation (difference). This difference is then divided by the calculated standard error unit to give the number of standard error units from the correct value. This is the number plotted on the appropriate horizontal line.

Assume, for example, the first serum SPRM analysis is run during a quarter, and a value of 105 ppb is obtained for the content of DDT. The spiking level, however, was 150 ppb. One standard error unit (SEU) is calculated by multiplication of the formulation value by the percent RSD to give a standard error unit that should be valid throughout the life of the specific SPRM: 150 x 0.10 = 15 ppb = one SEU. The difference 105 - 150 = 45 is then divided by 15 to give the number of standard error units to be plotted, in this case -3.0. If the second result is 125 ppb, the second point plotted along the horizontal axis would be calculated as:

$$\frac{125 - 150}{\text{one SEU}} \text{ or } \frac{-25}{15} = -1.7 \text{ SEU}$$

Figure 3-A. Laboratory A control curves for blood SPRM, three-month period.

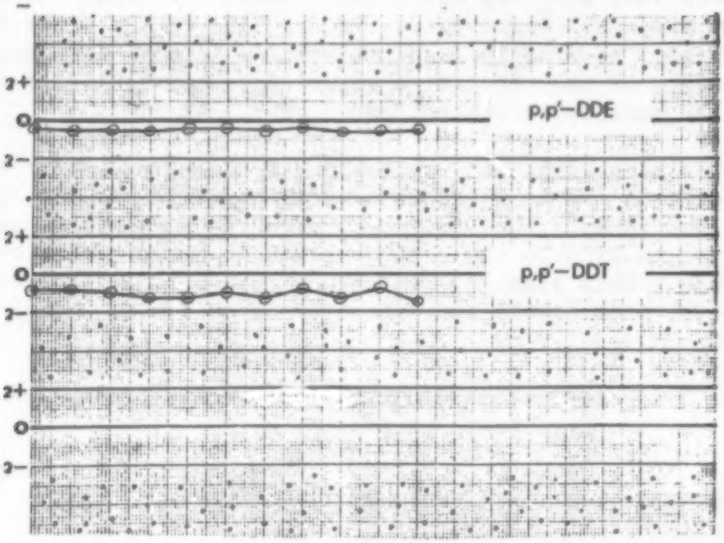
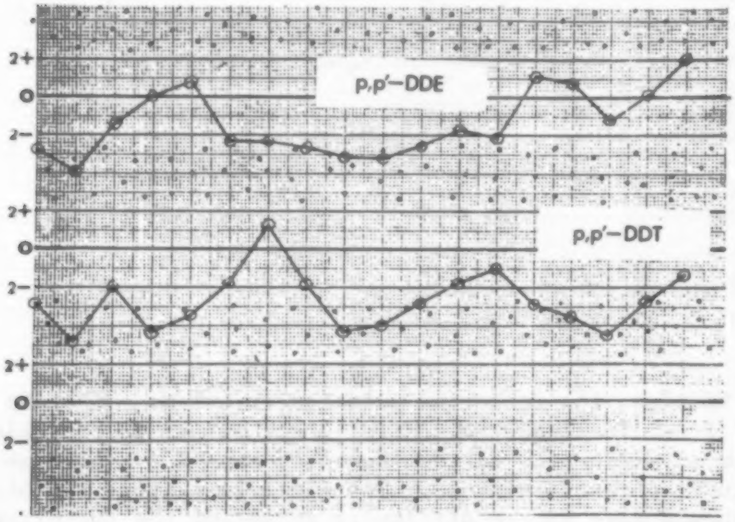


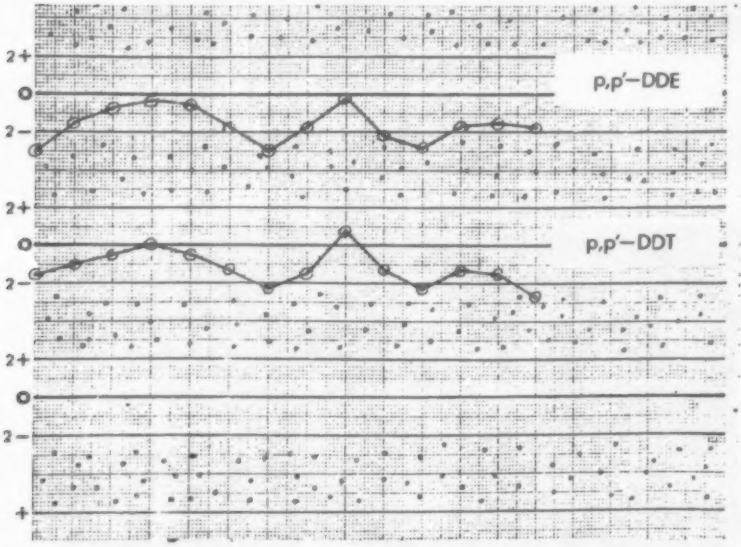
Figure 3-B. Laboratory B control curves for blood SPRM, three-month period.



When constructed in this way, quality control charts readily show levels of accuracy and precision for repetitive analyses by a given analyst. Figure 3-A demonstrates excellent precision since the results all fall along an essentially horizontal line. Accuracy is good because this line is well within the control area of ±2 SEU, all recovery values being slightly low, probably due to an inherent negative determinative error in the procedure being used. Figure 3-B, on the other hand, demonstrates very poor analytical performance in both accuracy and precision. Nine of the repetitive values for DDE and eleven for DDT are out of the acceptable control range of ±2 SEU.

Control charts also highlight cases where errors are present exerting similar effects on the analyses of several pesticides. The following Figure 3-C, for example, demonstrates rather poor precision and also a distinct correlation in the configuration or shape of the curves for both compounds. This signals some common error proportionately affecting both compounds, most likely the extraction step in this blood analysis.

Figure 3-C. Laboratory C control curves for blood SPRM, three-month period.



From time to time, the following question is asked; "What is to prevent an analyst from 'fudging' the control chart points so that his curves will appear significantly better than they should"? This can and has occurred in very rare instances. The alert laboratory administrator, however, should have little difficulty detecting the doctoring of curves. When a chart is submitted that is virtually a straight line such as that for p,p'-DDE in Figure 3-A, his suspicion should be aroused to the extent of personally checking the raw data to either confirm or refute his apprehensions. Furthermore, such an apparently outstanding performance will catch the attention of other analysts in the peer group whose data may look relatively poor by comparison.

In the first sentence of this subsection it was stated that "it may be desirable" to prepare control charts. One main value of the charts is to detect trends. Therefore, if a given SPRM sample is analyzed on an infrequent basis, a chart would serve little purpose as trends would not be evidenced. On the other hand, if a laboratory is monitoring a waterway, for example, for certain pesticides or other organic pollutants, the number of routine samples per month may be 100 samples or more. If the controlling SPRM is analyzed at the recommended minimum rate of one SPRM per 10 samples, this would amount to at least 10 SPRM analyses per month, a number sufficient to justify preparation of the chart.

3G BENEFITS OF THE IN-HOUSE SPRM PROGRAM

Analyzing in-house SPRM's will require a certain amount of man hours during which laboratory personnel cannot accomplish routine, productive analytical work. The time, effort, and expense spent on such a program has proven an invaluable investment, however, in the quality of analytical output in those laboratories involved. For example, chemists from regulatory laboratories are sometimes called upon to testify in a court case based upon their analytical results. If a chemist is armed with high quality analytical assurance data, the validity of his results on the sample(s) in question will be much more difficult to disprove, and the case will be that much stronger.

If a laboratory has a correctly functioning intralaboratory control program in effect, the morale of personnel is high, everyone has confidence in the routine data output, and interlaboratory check samples can be taken in stride and handled with little disruption of the normal work schedule. Since a higher volume of uncontrolled analytical data is obviously of much less value than a lower output of reliable results, time and effort must be allowed for each pesticide analytical laboratory that cares about valid results to conduct a proper quality control program.

Certain minimum requirements are necessary for the physical plant in which analyses are to be performed. Minimum considerations should include such factors as safety of personnel, reasonable temperature and humidity control, an adequate ventilation system, refrigerated storage areas for samples, facilities for an assembly line layout if large numbers of samples are processed, and an efficient glassware wash area. In addition, all necessary equipment for safety, sample preparation, analysis, and sample and data processing must be available.

The following subsections are intended to highlight a number of in-house factors that can lead to inaccurate analytical data in any laboratory and to present guidelines for avoiding these pitfalls. Further details of many of the areas mentioned will be given in appropriate later sections of this Manual or are covered in the cited sections of the EPA PAM.

3H ANALYTICAL BALANCES

Most laboratories contain balances of two types. Rough triple beam or Dial-O-Gram balances are used for weighing approximate amounts of materials to the nearest 0.01 or 0.001 g. For example, to prepare one liter of a 2 percent solution of NaCl for use in the liquid-liquid partitioning step of the modified Mills, Onley, Gaither Procedure [EPA PAM, Section 5,A,(1),(a)] the required 20 g of salt could be weighed out on one of these rough balances since the concentration of the solution is specified to only one significant figure.

An analytical balance is required, however, for the critical weighing of primary analytical pesticide standards in preparing standard reference solutions. The usual analytical balance has a capacity of 160 g and a capability of weighing to the nearest ±0.0001 g (error and uncertainty), the fourth decimal place being obtained by estimation and, therefore, the final significant figure recordable (Subsection 2Kb). This leads to a total accuracy and precision of

$$\frac{0.0002\ g}{0.0200\ g} \times 100 = 1\%$$

in weighing 20.0 mg of pesticide standard by difference (two weighings), as in usually done in preparing primary standard solutions (Subsection 30). This value is quite acceptable considering the other errors inherent in the total analytical scheme.

The accuracy, precision, and sensitivity of the analytical balance should be checked at least once a year by a qualified balance specialist, and the balance should be used properly by all personnel to insure its maintenance in good condition at all times. Since the analytical balance is used to weigh standard pesticides for preparation of solutions upon which all analytical results are based, its importance, and the need for its care and protection, should be obvious. The single pan, direct reading analytical balance that weighs by the principle of substitution is by far the type in widest use today. As compared with the classic, double pan, equal arm balance, the single pan balance is more automatic, convenient, and much faster (although no more accurate or precise), but it is still a very fragile instrument requiring certain precautions in its use. These include the following:

a. The balance should be placed on a heavy, shock proof table or cement block slab built up to convenient height from the floor.

b. The balance is preferably located away from laboratory traffic and protected from drafts and humidity changes.

c. The balance temperature, room temperature, and temperature of the object being weighed should be equilibrated.

d. When not in use the balance beam should be locked, objects removed from inside, and all weights released from the beam.

e. The inside and outside of the balance must be kept scrupulously clean. Never place chemicals directly on the balance pan. Remove spilled chemicals immediately with a brush.

Before using an analytical balance for the first time, the manufacturer's literature should be consulted or instructions obtained from someone experienced in its proper use.

3I PURITY OF SOLVENTS

The purity of reagents, solvents, adsorbents, distilled water, etc. is of extreme importance when analyzing samples for residues in the low ppm or ppb range. The electron capture detector senses any electron capturing materials in the injected sample, whether they be pesticides or other impurities. Quite often, extraneous contaminants will give rise to GC peaks that may precisely match the retention characteristics of certain pesticides, even on two or three different stationary phases. A common contaminant of solvents and reagents is di-*i*-butyl phthalate plasticizer, which can be easily confused with BHC and aldrin in GC with electron capture detection. Construction materials have been suggested as the source of phthalic acid esters and PCBs present in laboratory air and the cause of solvent, reagent, and glassware contamination (1). Sulfur and sulfur-containing compounds can be present in solvents and column materials, as well as in certain substrates (onion, cabbage, turnips), and can give rise to peaks easily confused with pesticides (2). The use of plastic gloves, Tygon tubing, plastic tubing, Nalgene containers, and plastic screw caps without Teflon liners should be strictly avoided whenever contact with organic solvents is possible.

Commercial solvents designated "pesticide grade" or "distilled in glass" can usually be used without further treatment, but care must be exercised in their storage. For example, it was reported that photo-chemical reactions can produce compounds from pesticide-grade hexane that are detected by an electron capture detector and interfere with pesticide residue determinations (3). Storage in the dark was recommended to prevent this. Reagent- or technical-grade solvents almost always require distillation by the user in an all-glass still. In any case, each solvent should be checked before use for interference in the analytical procedure by evaporating a portion to provide as great a concentration factor as will ever be employed in any method for which the solvent will be used.

A typical procedure is to concentrate 100 ml of solvent to 1 ml and to inject 5 µl into the gas chromatograph equipped with the detector of choice. Detector response is recorded for at least 20 to 30 minutes. No cloudiness or discoloration should be observed when the volume is reduced, and no GC peaks that would interfere with sample analysis should be produced. Details of the test for electron capture GC are given in Section 3,C of the EPA PAM.

Tests for interfering substances not detected by this procedure but causing pesticide degradation and loss are made by carrying known amounts of standards through the analytical method in the absence of any sample substrate (a complete reagent blank check). Solvents containing oxidants are especially troublesome in causing losses of organophosphorus pesticides, most notably carbophenothion. Acetonitrile and ethyl ether are two common solvents that may require special attention. Impure acetonitrile, the vapors of which will turn moistened red litmus paper blue when held over the mouth of the bottle, should be redistilled. Recoveries of some phosphate pesticides from Florisil columns are low if peroxides are present in ethyl ether eluants. Ethyl ether is tested for the presence of peroxides by adding 1 ml of fresh 10% KI solution to 10 ml of solvent in a clean glass-stoppered flask or cylinder previously rinsed with ether. Shake and let stand for 10 minutes. No yellow color should be observed in either layer. If present, peroxides are removed by extraction with water, after which the 2% ethanol normally present in ether and also removed by the partitioning is replaced [EPA PAM, Section 5,A,(1),(a)].

Reagent grade solvents purchased in large cans with plastic pour-spouts can be a significant source of contamination. If these solvents are used in the laboratory glassware cleaning routine, the glassware should be rinsed with pesticide grade solvent immediately after rinsing with the reagent grade solvent from the can. The solvent from these cans should never be used for extraction of samples or dilution of samples or standards. Metal safety cans commonly used for solvent storage can also contribute contamination. Plastic snap caps that seal cans of diethyl ether can be a large source of sample background. These impurities begin to elute early in the chromatogram and continue until well after the p,p'-DDT peak (relative retention of 4.6 on a 1.5% OV-17/1.95% OV-210 column).

Solvent purity for HPLC (4, 5) is at least as critical as for GC, and it is frequently necessary to repurify even the highest quality commercial solvents. Impure solvents can lead to baseline instability, spurious peaks, variable retention volumes, impure recovered fractions, and other problems. Solvent purity is more important in gradient than in isocratic elution. This is especially true of the weaker solvent since more of it passes through the column, and its impurities can be concentrated on the column head. Intentional impurities such as the ethanol stabilizer in chloroform and the antioxidant (UV absorbing) in tetrahydrofuran (THF), as well as HCl or oxidation products in chlorinated hydrocarbons, benzene in hexane, and the aforementioned peroxides in ethers, may have to be removed if they interfere. Water content of solvents has an important effect on separations and must be controlled. Tests for solvent purity include recording the UV spectrum in a 5 or 10 cm cell versus air over the dynamic range of the UV detector, spotting residue from evaporation of a large volume for TLC with I_2 vapor visualization, and Karl Fisher titration for water. Antioxidants are easily removed from THF by distillation, but the THF then rapidly oxidizes and must be tested for peroxides with KI as described earlier. HCl is removed from chlorinated solvents and alcohol from chloroform by extraction with water. Water and some other polar impurities are removed from low to moderately polar solvents by column chromatography on activated silica (heated to 175°C), alumina (heated to 300°C), or a molecular sieve. About 2-6 bed volumes should be passed through the adsorbent before replacing it; low cost, larger particle adsorbents that can be dry packed may be used. Water content of solvents is best controlled by preparing dry solvent and blending with water-saturated solvents. Impurities in water are removed by filtration, reverse osmosis, deionization, distillation (neat or from alkaline permanganate), electrolysis, passage through a reverse-phase column (for reverse-phase separations), or combinations of these.

Reagent grade water, especially purified for HPLC use, is commercially available from several sources. Particulates are removed from solvents (especially those cleaned up on an adsorbent column) prior to use in HPLC by passage through a solvent-resistant 0.5 μ membrane filter, and dissolved gases are removed by heating, stirring under a vacuum, or ultrasonic agitation. The composition of solvent mixtures can be altered by prolonged heating or exposure to vacuum. Table 3-3 summarizes some aspects of solvent purity in HPLC as outlined by one instrument manufacturer.

TABLE 3-3

SOLVENT PURITY IN LC [5]

Contaminant	Possible Source	Effect	Removal
Particulate matter (dust,etc)	During transfer, unclean vessels	May block in-line filters, lodge in pump seals, or accumulate at column head.	Filtration through membrane filter.
Water	Glassware, solvent preparation or manufacture	Variable column activity. k' variation, stability of silicate ester bonded phases.	Drying over molecular sieve or anhydrous sodium sulfate.
Alcohol	Stabiliser in chloroform, impurity in hydrocarbons	Similar to water.	From hydrocarbons, pass through activated silica; from $CHCl_3$ extract with H_2O, dry with Na_2SO_4.
Hydrocarbons (in water)	Organic matter	Baseline instability during gradient elution.	Passage through porous polymer column or C_{18} bonded phase.
Peroxides (in ethers)	Degradation	Oxidation of bonded phase (e.g., $-NH_2$ to $-NO_2$), reaction with sample, column deactivation or degradation (polystyrene-based).	Distillation or passage through activated silica gel or alumina.
HCl, HBr (halogenated solvents)	Degradation	Column degradation esp. bonded phases, UV absorbance (bromide), stainless steel attack.	Passage through activated silica or calcium carbonate chips.
BHT *	Antioxidant in THF	UV absorbing.	Distillation.
Dissolved oxygen	Solvent preparation	Degrades polystyrene-based packing, oxidizes β,β'-oxydipropionitrile, may react with sample.	Degas solvent with vacuum or heat.
Unknown UV-absorbing	From manufacture	Baseline instability or drift during gradient elution, high detector background.	Try activated silica or alumina; distillation for organics and recrystallization or passage over ion-exchange column for inorganics.
High boiling compounds	From solvent manufacture	Contaminates collected sample in preparative LC.	Distillation.
Algae in water	Growth during prolonged storage	Can plug in-line filters, column entrance frits.	Distillation from alkaline permanganate or discard.

* butyl hydroxytoluene

3J DISTILLATION OF SOLVENTS (6, 7)

Distill reagent grade acetonitrile over reagent grade $AgNO_3$ (3 g/l) with an all-glass fractionating column equipped with a water cooled condenser. Discard about the first 10% of the distillate and leave the last 20% of the solvent in the flask. Rinse the flask and use fresh $AgNO_3$ and boiling chips for each distillation. Test the distillate for interference. Alternatively, to 4 liters of acetonitrile add 1 ml of 85% H_3PO_4, 30 g P_2O_5, and boiling chips. Allow to stand overnight and then distill from all-glass apparatus at 81-82°C (do not exceed 82°C), discarding the first and last 10% of distillate. Distill acetone, hexane, benzene, carbon tetrachloride, chloroform, ethyl ether, isopropanol, methanol, methylene chloride, isooctane, petroleum ether, and ethyl acetate from all-glass apparatus. A technique for recovery of reusable solvent from Kuderna-Danish evaporators has been described (8).

3K CONTAMINATION FROM REAGENTS AND MATERIALS

Any other reagents used in the extraction or cleanup steps are also potential sources of contamination. These reagents, such as sodium sulfate (Na_2SO_4), glass beads, sodium chloride, and glass wool, should be pre-extracted with the solvent to be used in the analytical method or another solvent known to remove the potential interferences. For example, Na_2SO_4 is extracted in a reserved Soxhlet apparatus, the thimble of which is pre-extracted before the first use. Methanol followed by hexane or petroleum ether are cycled for several hours each, after which the Na_2SO_4 is dried and stored in a glass container with a glass cap at 130°C in the oven used to dry Florisil and other adsorbents. Plastic fiber pack liners have been found to contribute PCBs and phthalates to Na_2SO_4 that must be removed by this procedure. Phthalate esters are also removed from sodium sulfate by heating at 600°C for 4 hours in a muffle furnace (FDA PAM, Section 121). Impurities in batches of silicic acid that interfere with separations of pesticides from PCBs were reduced by extraction of the adsorbent with solvent (9). Adsorbents that are activated and stored in an oven that is not cleaned at least yearly will absorb vapors from the oven. These impurities may be eluted along with pesticides in cleanup procedures and could interfere in the later determinative step.

Filter paper and other reagents and apparatus should be checked by washing through the solvent to be used and injecting a sample, after concentration, into the gas chromatograph. No peaks should appear. Impurities from filter paper were the cause of interfering signals in the GC-alkali flame ionization detector determination of pesticide residues in plants; Soxhlet pre-extraction of the paper with acetone was recommended (10). Teflon and aluminum foil should be rinsed with an appropriate solvent. Solvents in polyethylene wash bottles can become contaminated with electron capturing and UV absorbing species and should be tested for impurities. Better still, avoid the use of plastic wash bottles and use Teflon or all-glass ones. Glass wool was shown to contain hydrocarbons, phthalate esters, and unesterified acids, among other compounds detected by GC. The most efficient way of eliminating these impurities was to treat the wool for a few minutes with hydrogen chloride vapors, followed by continuous Soxhlet extraction for 24 hours with methylene chloride (11). Losses of 2,4-D caused by a glass wool plug have been reported (12).

Distilled water can be troublesome, particularly in a procedure where a large volume is used. Such a procedure is the Mills, Onley, Gaither cleanup method for adipose tissue [EPA PAM, Section 5,A,(1),(a)] where 700 ml of water is partitioned with acetonitrile, the latter being finally concentrated to 5 ml (a potential contaminant concentration factor of 700/5 = 140). Since the source of contamination in laboratory water is organic in nature, distillation will not be sufficient cleanup if the organic material co-distills with the water. An activated charcoal filtration prior to the distillation procedure has been found to significantly improve water quality. If deionization through a column of ion exchange resin is carried out, an activated charcoal filter should be installed between the column and the distillation equipment to trap any organic impurities eluted from the resin before the water enters the still. For analyses at ppb and ppt levels, distilled and deionized water should be further purified by a double extraction with an appropriate immiscible solvent, e.g., benzene or isooctane, followed by boiling, if necessary, to remove the residual solvent. Aqueous salt solutions such as 2% NaCl or saturated NaCl used in some isolation procedures are prepared from properly purified salt and water and then solvent extracted as a further precaution. Contamination can result from Teflon or Tygon lines and/or plastic resin or charcoal cartridges. Water samples from systems containing these elements must be analyzed at the level of sensitivity necessary for the analysis prior to use of the water.

Materials in which the initial sample is stored must be given consideration. Polyethylene bags are totally unsuitable for samples to be examined by electron capture GC or TLC because of trace contaminants that may be present. As an example, it has been reported (2) that polyethylene contains a contaminant that reacts with $AgNO_3$ chromogenic reagent, giving a TLC spot close to that of p,p'-DDE and having similar GC retention times to o,p'-DDE and p,p'-DDE. Figure 3-D shows a gas chromatogram of a hexane extract of the cardboard liner from a common type screw-cap bottle. The peaks were found by GC-MS to be due to various phthalates. Although these plasticizers would not interfere with GC if a halogen-selective detector was used, they are a potential interference for electron capture-GC and GC-MS analysis (13). Glass containers with solvent-rinsed aluminum foil or Teflon-lined caps are generally acceptable as sample containers and for storing purified reagents.

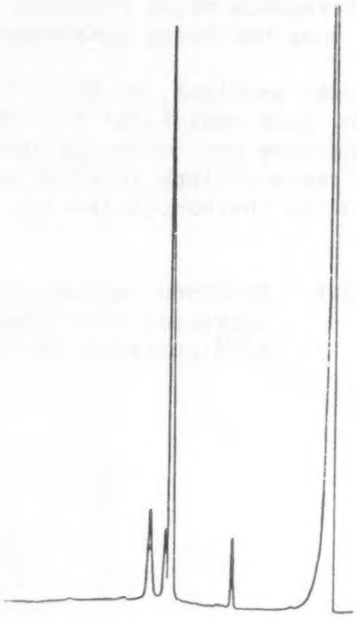


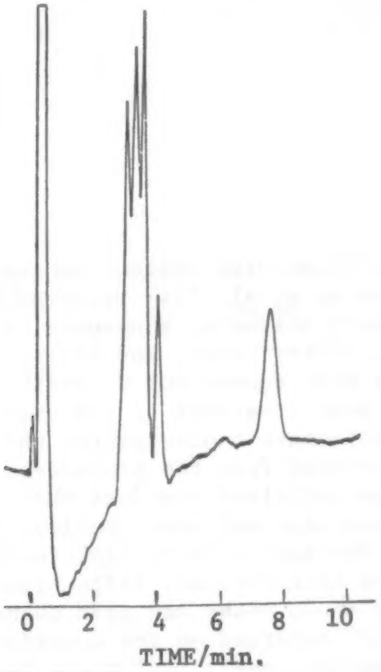
Figure 3-D. Gas chromatography of hexane extract of cardboard bottle cap liner on 10% OV-3 column with a hydrogen flame ionization detector at 16 x 10^{-11} amp/mV sensitivity. Amount injected represents 1/100 of the extract from one 3/4 inch diameter liner.

Other examples of problems with reagent contaminants have appeared in the literature. Bevenue et al. (14) reported on the contribution of contaminants by organic solvents, glassware, plastic ware, cellulose extraction thimbles, filter paper, and silica gels to water samples causing interference with subsequent GC analysis in the ppb range. Prior to their use, heat treatment of glassware and the silica gels was recommended to eliminate contaminants, while plastic ware and filter paper were excluded from the procedure. Levi and Nowicki (15) found that cloth bags contained residues that were absorbed by cereal grains stored in these bags and gave spurious GC peaks with electron capture detection. The same workers (16) found that Na_2SO_4, filter papers, solvents from wash bottles, Teflon gaskets, and glass wool produced interfering EC-GC peaks and gave methods for their elimination. Bevenue and Ogata (17) reported on the contribution of extraneous components by high purity, analytical grade basic reagents used for adjustment of pH during isolation steps in the analysis of chlorophenoxy acid esters and ethyl or methyl derivatives of hexachlorophene and PCP in plant and animal tissue and water samples. Baker et al. (18) found contamination of acetone with an impurity corresponding to CCl_4 and interfering in the analysis of the latter pesticide (fumigant) by EC-GC. It was shown that this contamination could be caused by CCl_4 in the laboratory atmosphere, possibly arising from the use of aerosol propellant cans for spraying thin layer chromatograms. Trotter and Young (19) found that impurities in $SbCl_5$ reagent caused erratic recoveries of PCBs in perchlorination procedures.

The chromatogram in Figure 3-E is of material extracted from disposable vinyl laboratory gloves. A chemist wearing these gloves had touched the lip of a concentrator tube with the fingertip of his gloved hand. One of the extraneous peaks produced coincided exactly with the compound (TCDD) that was being determined.

In view of these problems, it is mandatory that reagent blanks be run constantly for each analytical procedure, with final extracts being reduced to the same concentration level normally used for the sample material. A reagent blank involves repetition of the entire procedure without including the sample itself.

Figure 3-E. Electron capture gas chromatogram of material extracted with n-hexane from outside surfaces of disposable vinyl plastic laboratory gloves.



3L GC RETENTION DATA FOR COMMON INTERFERENCES

Table 3-4 contains relative retention data for common contaminants on several GC liquid phases used in EPA and FDA laboratories (Section 5L). These compounds will be ~~eluted at~~ the same positions as certain pesticides (EPA PAM, Section 4) and will, therefore, interfere in the analysis of the pesticides or be mistaken for them.

Table 3-4

GC RETENTION DATA FOR COMPOUNDS COMMONLY INTERFERING WITH PESTICIDE ANALYSIS

	Relative Retention Time				
	EPA Columns			FDA Columns	
Liquid Phase	1.5% OV-17/ 1.95% OV-210	4% SE-30/ 6% OV-210	5% OV-210	10% DC-200	5% DC-200/ 7.5% QF-1
Oven Temperature	200°C	200°C	180°C	200°C	200°C
Column Dimensions	1.8 m x 4 mm	1.8 m x 4 mm	1.8 m x 4 mm	1.8mm x 4 mm	1.8 m x 4 mm
Flow Rate	60 ml /min	70 ml/min	50 ml/min	120 ml/min	120 ml/min
Compounds					
Phthalates a/					
Dimethyl phthalate	0.30	0.30	0.52	0.14	0.26
Diethyl phthalate	0.41	0.42	0.72		
Dibutyl phthalate	1.22	1.18	2.06	0.85	1.20
Di-iso-butyl phthalate	0.86	0.91	1.50	0.64	0.88
Diamyl phthalate	2.19	2.11	3.43		
Butylbenzyl phthalate	5.49	4.00	7.2	3.00	4.00
Di-(2-ethylhexyl) phthalate	7.3	6.6	11.4	6.4	6.6
Dioctyl phthalate	14.6	12.2	18.6	11.8	11.9
Diphenyl phthalate	12.7	7.7	17.8		
Other dicarboxylic acid esters a,b/					
Di-iso-butyl adipate	<1 c/				
Di-(2-ethylhexyl) adipate	3.66				
Di-(2-ethylhexyl) azelate	11.0				
Dibutyl sebacate	5.14				
Di-(2-ethylhexyl) sebacate	15.6				
Chlorinated compounds (from Nalgene)					
Hexachlorobenzene	0.48	0.45	0.46	0.43	0.42
Phosphates d/					
Tributoxyethyl phosphate	3.82	3.07	2.50		

a/ Retention relative to aldrin
b/ Flame ionization detector used because of lack of sensitivity with electron capture detector
c/ Elutes with the solvent peak
d/ Retention relative to ethyl parathion

3M CLEANING OF GLASSWARE

The residue analytical chemist must be sure his glassware is entirely free from contamination. The cleaning operation generally includes:

a. Soaking and washing in a high temperature (50°C) bath of synthetic detergent (e.g., Alconox) in water.

b. Rinsing with tap water.

c. Rinsing with distilled water.

d. Rinsing with acetone.

Cleaning of glassware used to concentrate samples (e.g., K-D flasks or evaporative concentrator tubes) should include a soak for at least 15 minutes in hot (40-50°C) chromic acid cleaning solution (observe rigid safety precautions) after the tap water rinse to remove all traces of organic material. This soak is followed by thorough rinsing with tap and distilled water and then with acetone and hexane. Pipets are washed in the same way, preferably using a commercial automatic or semiautomatic self-contained washing unit.

Large glass items such as beakers and flasks are inverted and suspended to dry in metal racks. Small items such as glass stoppers and bottle caps are wrapped in aluminum foil, dried in an oven, and stored in foil. Pipets are wrapped in bundles in aluminum foil and oven dried.

Clean, dry glassware is stored in a dust-free cabinet. (Stainless steel storage tubes are available for pipets). As an extra precaution, each piece should be rinsed with the solvent to be employed in the analysis immediately before use. As soon as possible after a piece of glassware has come in contact with a sample containing pesticides, it should be rinsed with acetone to remove surface residues. If this is not done, the subsequent soak bath of detergent will pick up the pesticide and may then serve to contaminate all other glassware placed therein. Details for cleaning glassware are given in the EPA PAM, Section 3,A.

3N HOUSEKEEPING

Good general housekeeping procedures are important in the analytical laboratory. Benches should be neat, labels legible, and files orderly. Certain contaminants such as cleaning agents and dust are impossible to exclude, but others should not be deliberately introduced, such as by eating or smoking in the laboratory. To reduce possibilities of errors and cross contamination, food, beverages, or snacks should not be stored in a refrigerator used to store samples.

30 ANALYTICAL PESTICIDE REFERENCE STANDARDS

It has often been noted when evaluating chromatograms from interlaboratory check samples that reference standards used in certain laboratories reporting rejected results were undoubtedly inaccurate. (This can be determined by the coordinator by comparing the peak height ratios in the chromatograms from the check sample of precisely known composition against the same ratios from the laboratories' internal standards). The proper preparation and storage of analytical standard solutions is of utmost importance. Since the working, diluted standards may be in use for up to six months, any mistakes in preparation of the concentrated stock solutions or in their dilution would be reflected in the accuracy of analytical results for this entire period. Incorrect standards will result in correspondingly incorrect analytical data even though first class technique is thereafter employed and all laboratory instruments are in perfect operating condition. Even including improperly operated equipment, the greatest single source of quantitative error in GC analysis is undoubtedly inaccurate standard solutions.

Identification and record keeping of reference standard solutions are activities that often receive too little attention in some laboratories. Its importance cannot be overemphasized, particularly in a laboratory concerned with law enforcement. Therefore, the protocol should be formalized and standardized for all staff chemists within the laboratory group. By so doing, it should be possible for any other staff chemist or a supervisor to consult a given chemist's reference standards logbook years after an analysis was conducted and readily determine the precise identity and concentration of any standard used in an analysis.

The logbook should reflect a complete record of each prepared reference standard solution, starting with the pure primary standard and ending with the final working standard solution. Data that should be documented include weight of primary standard, concentration of all subsequent serial dilutions, and the dates of preparation of all dilutions.

In multiresidue analysis, it is common practice to prepare final working standards as a mixture of pesticides of interest to the laboratory, this subject to be treated in some detail later in this section. Such a mixture should be assigned an identification number and so documented in the logbook. The same number should be printed on the bottle label of the mixture and should also be used to identify all reference standard chromatograms during the life of the mixture.

In one possible coding system, each standard is assigned a number preceded by the letter C for "concentrated", I for "intermediate", and W for "working". Referring to Figure 3-I, the concentrated stock solutions could be given the numbers C1 (lindane), C2 (aldrin), C3 (dieldrin), C4 (*o*,*p*'-DDT), and C5 (*p*,*p*'-DDT). The number would represent the compound, and the prefix the stage of concentration. After dilution, the intermediate stock solutions would be designated I1, I2, I3, I4, and I5, respectively. The final working standard mixture prepared from these solutions could be designated W1-5, or it can be given a totally new number such as W6. The latter is probably less awkward in certain situations, e.g., if the final working standard mixture is remade using solutions I1-I4 and a later-prepared standard of p,p'-DDT (perhaps designated I10). The new working standard would be designated W1-4,10 with the former system, but could be numbered more simply as W7 if a unique sequential system number is given to each solution. It is likely that each laboratory can devise a numbering system to suit its needs. The important point is to use some clear and consistent system to designate standards and to have records fully describing the preparation of each numbered solution. Sample sheets for maintenance of the reference standards logbook are shown in Figures 3-F, 3-G, and 3-H. These forms are in routine use at the EPA laboratory in Research Triangle Park, NC.

Organic compounds are subject to a wide variety of oxidation, hydrolysis, isomerization, and polymerization reactions. Instability of organic standards is, therefore, often a problem. Storage and use conditions should be those that retard degradative processes, and purity should be periodically rechecked.

Details for the preparation, storage, and use of pesticide analytical standards are given in Section 3,B of the EPA PAM. Some important considerations as they pertain to quality control and identification of potential trouble spots are outlined below.

a. Primary Standards

There are no officially recognized pesticide "primary" standards, although in the parlance of the pesticide chemist, analytical grade standards of 99% or higher purity are referred to as primary standards. Purities of standards are commonly greater than 99% and seldom less than 95% but may be lower in some cases. For example, chlordane and toxaphene are available in technical grade with 60-70% purity. The percentage of purity must be known in order to apply a correction factor in weighing out the standard for subsequent dilution.

There are several sources of pesticide standards. Most manufacturing companies will supply the analyst with technical grade pesticides and in some cases a small amount of a more highly purified grade. The technical material may be purified by repeated recrystallization and checked for purity by at least two analytical criteria such as elemental composition; IR, NMR, or mass spectrum; melting point; GC trace; or TLC spot pattern. The EPA Quality Assurance Program maintains a pesticide calibration and reference materials repository at its Pesticide Laboratory at Research Triangle Park, NC. This laboratory supplies 100 mg or less of standards of certain pesticides, metabolites, and derivatives, on a discretionary basis as time and resources permit, to nonprofit, government, and university laboratories. EPA publication EPA-600/9-78-012

Figure 3-F

PREPARATION OF CONCENTRATED STOCK STANDARDS

No.____ Date __/__/__ Chemist ________

Compound ________ Lot No. ____ Purity ____ %

Final Gross Wt ____ g Solvent ________

*Tare Wt ____ g Dilution Vol. ____ ml

Net Wt ____ g Concentr. ____ ng/µl

**Adj. Net Wt ____ mg

No.____ Date __/__/__ Chemist ________

Compound ________ Lot No. ____ Purity ____ %

Final Gross Wt ____ g Solvent ________

*Tare Wt ____ g Dilution Vol. ____ ml

Net Wt ____ g Concentr. ____ ng/µl

**Adj. Net Wt ____ mg

No.____ Date __/__/__ Chemist ________

Compound ________ Lot No. ____ Purity ____ %

Final Gross Wt ____ g Solvent ________

*Tare Wt ____ g Dilution Vol. ____ ml

Net Wt ____ g Concentr. ____ ng/µl

**Adj. Net Wt ____ mg

No.____ Date __/__/__ Chemist ________

Compound ________ Lot No. ____ Purity ____ %

Final Gross Wt ____ g Solvent ________

*Tare Wt ____ g Dilution Vol ____ ml

Net Wt ____ g Concentr. ____ ng/µl

**Adj. Net Wt ____ mg

---

*If weighing into a beaker, this is the empty beaker weight. If weighing from a dropping bottle, this is the initial weight of bottle and contents.

**Corrected for purity of primary standard.

Figure 3-G

PREPARATION OF STANDARDS OF INTERMEDIARY CONCENTRATION

NO.______ Date___/___/___ Chemist________________

Compound________________________

Strength of Concentrated Stock__________ng/µl

Aliquot of Concentrated Stock__________ml

Dilution Volume__________ml

Dilution solvent__________

Final Concentration__________ng/µl

NO.______ Date___/___/___ Chemist________________

Compound________________________

Strength of Concentrated Stock__________ng/µl

Aliquot of Concentrated Stock__________ml

Dilution Volume__________ml

Dilution solvent__________

Final Concentration__________ng/µl

NO.______ Date___/___/___ Chemist________________

Compound________________________

Strength of Concentrated Stock__________ng/µl

Aliquot of Concentrated Stock__________ml

Dilution Volume__________ml

Dilution solvent__________

Final Concentration__________ng/µl

NO.______ Date___/___/___ Chemist________________

Compound________________________

Strength of Concentration Stock__________ng/µl

Aliquot of Concentrated Stock__________ml

Dilution Volume__________ml

Dilution solvent__________

Final Concentration__________ng/µl

Figure 3-H

PREPARATION OF FINAL WORKING STANDARD SOLUTIONS

No._____ Date___/___/___ Chemist__________

Solvent__________

	Compound	Parent Sol. Number	Conc. of Parent Sol. ng/µl	Aliq. Vol. ml	Dilution Vol. (ml)	Final Conc. pg/µl
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						

No._____ Date___/___/___ Chemist__________

Solvent__________

	Compound	Parent Sol. Number	Conc. of Parent Sol. ng/µl	Aliq. Vol. ml	Dilution Vol. (ml)	Final Conc. pg/µl
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						

No._____ Date___/___/___ Chemist__________

Solvent__________

	Compound	Parent Sol. Number	Conc. of Parent Sol. ng/µl	Aliq. Vol. ml	Dilution Vol. (ml)	Final Conc. pg/µl
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						

lists available standards and supplemental data. Purified standards can be purchased from a number of U.S. companies handling chromatographic equipment and supplies and from the National Physical Laboratory, Ministry of Technology, Chemical Standards, Teddington, Middlesex, England. Purities of these standards are not always what they are advertised to be, so the chemist should always verify the purity and repurify if necessary and practical.

Concentrated stock standard solutions are conveniently made up at a 200 ng/µl concentration by weighing 20.0 mg of pure standard and diluting to 100 ml. If the primary standard is given as 99.0 percent pure, weigh $\frac{20.0}{0.990}$ or 20.2 mg; if the purity is given as 90.0%, the weight will be

$$\frac{20.0}{0.900} = 22.2 \text{ mg.}$$

Toxicity levels and relative stabilities are important factors that dictate the methods of handling and storing various pesticide standards. Highly toxic pesticides (low LD_{50} values) require special precautions such as wearing disposable rubber or plastic gloves and avoiding inhalation of vapors. The stable organochlorine compounds may be stored at room temperature in tightly sealed containers, while organophosphates, which are subject to a wide variety of oxidations, rearrangements and hydrolytic reactions, should be desiccated in a refrigerator and allowed to come to room temperature in the desiccator before use. If standards are stored in a freezer, containers are not opened until warmed to room temperature, or condensed water vapor will be introduced.

b. Concentrated Stock Standards

Secondary standards are liquid solutions of the primary standards. The final concentration of working standard will depend upon its use, e.g., pg range for electron capture GC, ng range for TLC and other GC detectors, and µg range for IR spectroscopy.

For electron capture GC, usually three dilutions of the primary standard are made to arrive at the working standard. An analytical balance capable of weighing to at least 0.0001 g and scrupulously clean glassware are employed. Stable, low toxicity pesticides may be weighed into a small beaker or cupped aluminum foil, transferring solid compounds to the balance with a stainless steel micro spatula and liquids with a pipet or dropper. Crystalline standards weighed on aluminum foil are transferred dry through a small glass funnel into a volumetric flask, the foil and funnel being carefully rinsed with solvent. Standards weighed into beakers are completely dissolved (observe carefully) in a small volume of solvent and transferred quantitatively by rinsing with the rest of the solvent through the funnel into the volumetric flask. Liquid primary standards can alternatively be transferred to a dropping bottle with ground-in stopper; the bottle containing the standard is weighed, an estimated amount of standard transferred directly into a volumetric flask, and the bottle reweighed, the loss in weight representing the net sample weight. This closed dropping bottle technique is mandatory for high toxicity liquid pesticides. Solid primary standards may be weighed (10.0 mg) directly into 50 ml volumetric flasks, which will fit onto the pan of most one pan analytical balances. A procedure for storage and transfer of degradable pesticides under inert atmosphere is given in the FDA Pesticide Analytical Manual, Volume I, Section 132.

It is difficult with any of these techniques to weigh exactly the predetermined amount to obtain all solutions of 200 ng/µl. It is seldom necessary to take the trouble to attempt this, in any case, since the important thing is to obtain a formulation near to that which is desired and to know its exact value. This is calculated by dividing the known weight by the flask capacity. A possible procedure for preparing standards of exactly a certain concentration is to weigh the solid and then add only enough solvent (e.g., the solvent is measured from a pipet, or the solid is weighed into a graduated centrifuge tube and solvent added to the appropriate line) to give the desired concentration.

c. Intermediate Concentration Standards

It is usually necessary to prepare standards of intermediate concentration by dilution of the concentrated standards and then to prepare working standards by dilution of the intermediate standards. It is impractical and hazardous to prepare the final solution from the concentrated standard in one dilution or to prepare an original secondary standard at a concentration low enough to allow only one subsequent dilution. Some analysts have attempted to make this enormous dilution by aliquoting microliter volumes with a syringe into a volumetric flask. This is extremely poor technique, however, since an error of as little as 0.2 µl in a 5.0 µl transfer will be grossly magnified when a 5 to 10 µl injection of the resulting solution is chromatographed.

Separate solutions of each compound or a standard mixture can be prepared at this point. The concentration level depends on the response of the detection mode of the analytical procedure in which the standard will be used.

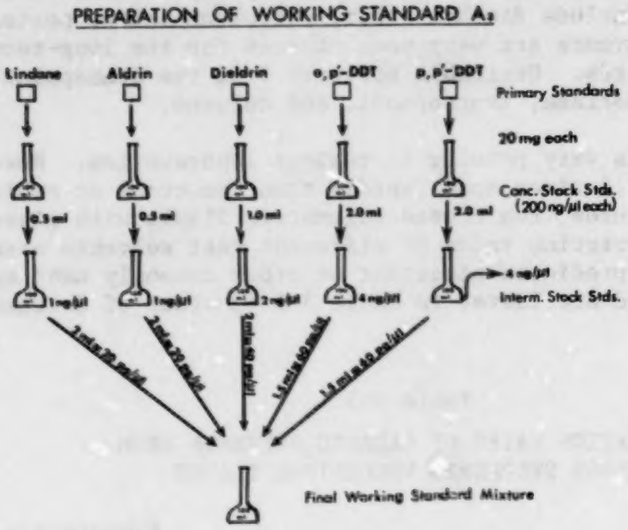
All solutions should be equilibrated to room temperature before any pipeting or diluting is carried out. Volumetric transfer pipets should be used where available, or a Mohr-type measuring pipet in other cases. Be sure to note whether the pipet is calibrated "to deliver" (TD) or "to contain" (TC) and use accordingly. The accuracy of well cared for, properly cleaned commercial Class A pipets and volumetric flasks is such that calibration is not required in order for potential errors from this source to be insignificant.

Pipets calibrated to deliver their stated volume should be used if possible. Measuring pipets are calibrated, like a buret, but do not deliver a volume of liquid as accurately or reproducibly as volumetric pipets. The latter are recommended whenever possible for analytical work. Pipets are filled by use of a rubber suction bulb rather than the mouth. After filling and dropping the meniscus to the etched line, no air bubbles should be evident anywhere in the pipet. The outside of the pipet tip is wiped free of liquid and the tip then placed against the inside wall of the vessel to which the solution is to be transferred. The liquid is discharged, keeping the tip against the inside for 20 seconds after the pipet has emptied. The pipet is removed from the side of the container with a rotating motion to completely discharge any drop on the tip. The small quantity of liquid inside the tip is not to be blown out; the pipet has been calibrated to account for this. Only properly cleaned and dried pipets can be inserted into the solution container without fear of contamination or dilution.

d. Working Standards

Working standards are generally made up as mixtures, the actual combinations being dependent upon the compounds of interest and the ability of the analytical method to resolve them. Care must be exercised that these mixtures do not contain compounds that may react with each other. Each working standard mixture should be made up at two or even three concentration levels, depending on variations in pesticide concentrations in routine samples. No compound should be present in such concentration that when injected into the gas chromatograph the linear range of the detector will be violated. If p,p'-DDT is present in a standard mixture, neither p,p'-DDD nor p,p'-DDE should be present since these compounds are breakdown products of DDT and their presence or absence is useful for monitoring this degradation. All compounds present in each mixture should be resolved by the working GC columns used in the laboratory. Suggested mixtures and concentration levels of common chlorinated pesticides for laboratories analyzing tissue samples by EC-GC with the recommended columns (Subsection 5L) are given in the EPA PAM, Section 3,B. A typical mixture, diagrammed in Figure 3-I, may be prepared as follows: weigh 20.0 mg each of primary standard lindane, aldrin, dieldrin, o,p'-DDT, and p,p'-DDT into separate 100 ml volumetric flasks to prepare concentrated stock solutions of 200 ng/μl each. Transfer, respectively, 0.5, 0.5, 1.0, 2.0 and 2.0 ml of each of these by separate pipets to individual 100 ml volumetric flasks to prepare intermediate stock standards. Transfer 2.0 ml of lindane, aldrin, and dieldrin and 1.5 ml each of o,p'-DDT and p,p'-DDT to the same 100 ml flask to prepare a final working standard mixture containing, respectively, 20, 20, 40, 60, and 60 pg/μl. For other than EC-GC, stock standards of 0.5 mg/ml and working standards from 50-100 to 0.5-1 ng/μl are typical. The procedure outlined in Figure 3-I is but one option for preparing the required solution; other approaches requiring less glassware are possible.

Figure 3-I. Serial dilutions of pesticide standard mixture


PREPARATION OF WORKING STANDARD A₁
Lindane
Aldrin
Dieldrin
o,p'-DDT
p,p'-DDT
Primary Standards
20 mg each
Conc. Stock Stds. (200 ng/µl each)
0.5 ml
0.5 ml
1.0 ml
2.0 ml
2.0 ml
1 ng/µl
1 ng/µl
2 ng/µl
4 ng/µl
4 ng/µl
Interm. Stock Stds.
Final Working Standard Mixture

e. Choice of Solvents and Storage of Standard Solutions

The EPA ETD laboratory has evaluated storage conditions for analytical reference standard solutions to minimize the decomposition of the reference standard in the solution and the evaporation of the solvent from the solution.

(1) Solvent Evaporation Control

The evaporation of the solvent containing the standard is easy to evaluate and observe. Under almost all conditions, some solvent evaporation can be measured. This solvent evaporation must be minimized to maintain the concentration of the standard solution for a reasonable length of time. The study of solvent evaporation centered around the following factors:

choice of solvent

solution volume

choice of container

storage temperature

(a) Choice of solvent

The chosen solvent should easily dissolve the reference standard, not chemically alter the reference standard, be compatible with the method of analysis, and not evaporate very rapidly. Solvents that evaporate rapidly include diethyl ether, petroleum ether, pentane, and acetone. These solvents are very poor choices for the long-term storage of reference standards. Desirable solvents from the standpoint of evaporation are isooctane, isopropanol, and toluene.

The use of hexane is very popular in residue laboratories. However, hexane evaporates 2.6 times more rapidly than isooctane at ambient laboratory temperatures from closed volumetric flasks with glass stoppers. The evaporation rates of different test solvents under these conditions and the predicted placement of other commonly used solvents that were not tested are listed in Table 3-5 in order of decreasing evaporation rate.

Table 3-5

EVAPORATION RATES OF ORGANIC SOLVENTS FROM GLASS STOPPERED VOLUMETRIC FLASKS

Solvent	Evaporation Rate (ml/wk)
Pentane	--
Diethyl ether	0.634
Methylene chloride	0.254
Acetone	0.221
Hexane	0.158
Chloroform	--
Benzene	0.096
Methanol	0.086
Ethyl acetate	--
Acetonitrile	--
Ethanol	--
Isooctane	0.061
Heptane	--
Isopropanol	--
Toluene	0.045
Decane	--

(b) Solution volume

Volumetric flasks of four different sizes were evaluated as to the effect of solution volume on the solvent evaporation rate. The absolute evaporation rates of hexane from the flasks (5 ml, 10 ml, 50 ml, and 100 ml) were 0.167, 0.155, 0.113, and 0.100 ml/week, respectively. The decreasing evaporation rates with increasing solution volume in itself makes the larger volume flasks more desirable. The relative evaporation rates (percentage of the solution volume evaporated per week) of the four flasks were 3.34, 1.55, 0.226, and 0.10% of the container volume evaporated per week, respectively. As GC measurements are sensitive to 3% volume changes, the use of the 5 and 10 ml volumetric flasks for more than one week is not recommended. The use of the larger solution volume increases the useful lifetime of the reference standards.

(c) Choice of container

Several different types of glassware were evaluated at ambient temperature in an attempt to find a container that is easy to use and will retard solvent evaporation. The containers tested included:

Volumetric flasks with glass $\overset{T}{S}$ stoppers

Screw-cap prescription bottles with cardboard lined plastic caps (Brockway Glass Co., Inc., Brockway, PA) with added Teflon disc cap liners (A. H. Thomas, #2390H)

Multivials (Supelco, Inc., #3-4579), 10 ml size

Serum bottles (Wheaton Scientific #223739), with Teflon faced septa (Wheaton Scientific #224167) and metal seal (Wheaton Scientific #224182).

Small volume flat bottom and conical vials from various sources with appropriate caps (Supelco, Inc., #3-3291, 3-3293, and 3-3300; Wheaton Scientific #225170 and 224882), 0.3, 0.6, and 1.0 ml sizes

Figures 3-J and 3-K illustrate these containers. Both hexane and isooctane were used as solvents in this evaluation, the results of which are summarized in Figure 3-L.

The evaporation rate of hexane from the volumetric flasks was the fastest found in this study. The use of volumetric flasks to store hexane solutions is discouraged. Isooctane also evaporated quite rapidly from volumetric flasks. The evaporation rates of hexane and isooctane are significantly reduced in screw-cap prescription bottles when compared to the volumetric flasks. The evaporation rates of the solvents from the prescription bottles was approximately 0.025 ml solvent evaporated per week. This rate is low enough to allow use of these containers.

Figure 3-J. Serum vial with Teflon-lined septum

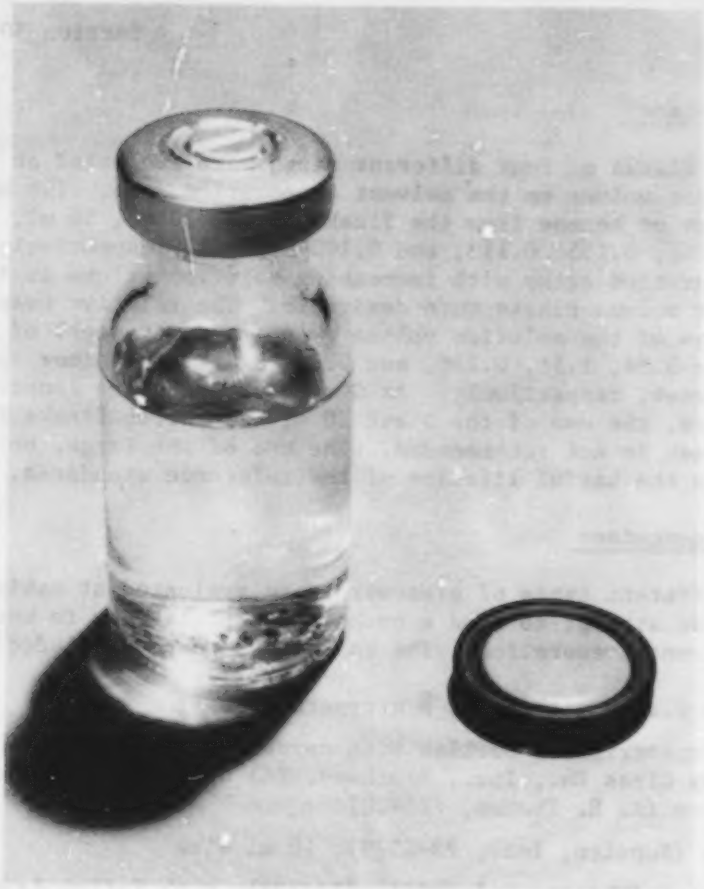


Figure 3-K. Other containers used in evaporation study. Back row (left to right): prescription bottle, volumetric flask, multivial. Front row. flat bottom vial, conical vial, conical vial.

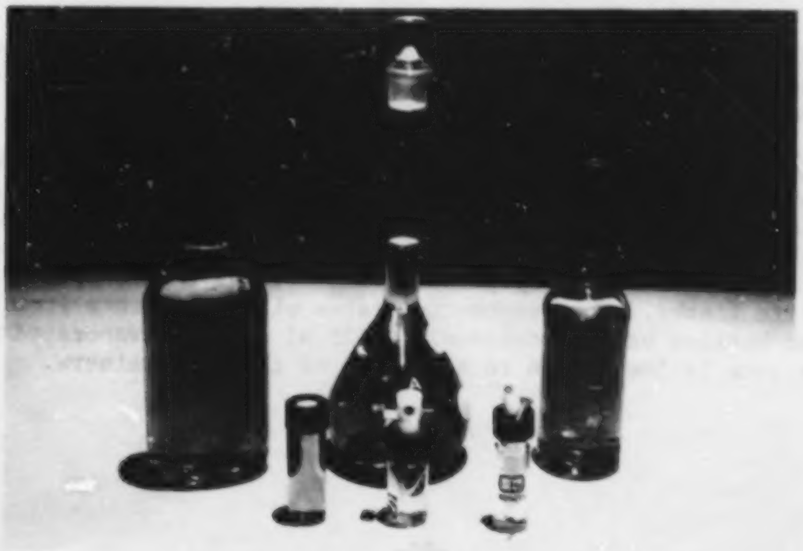
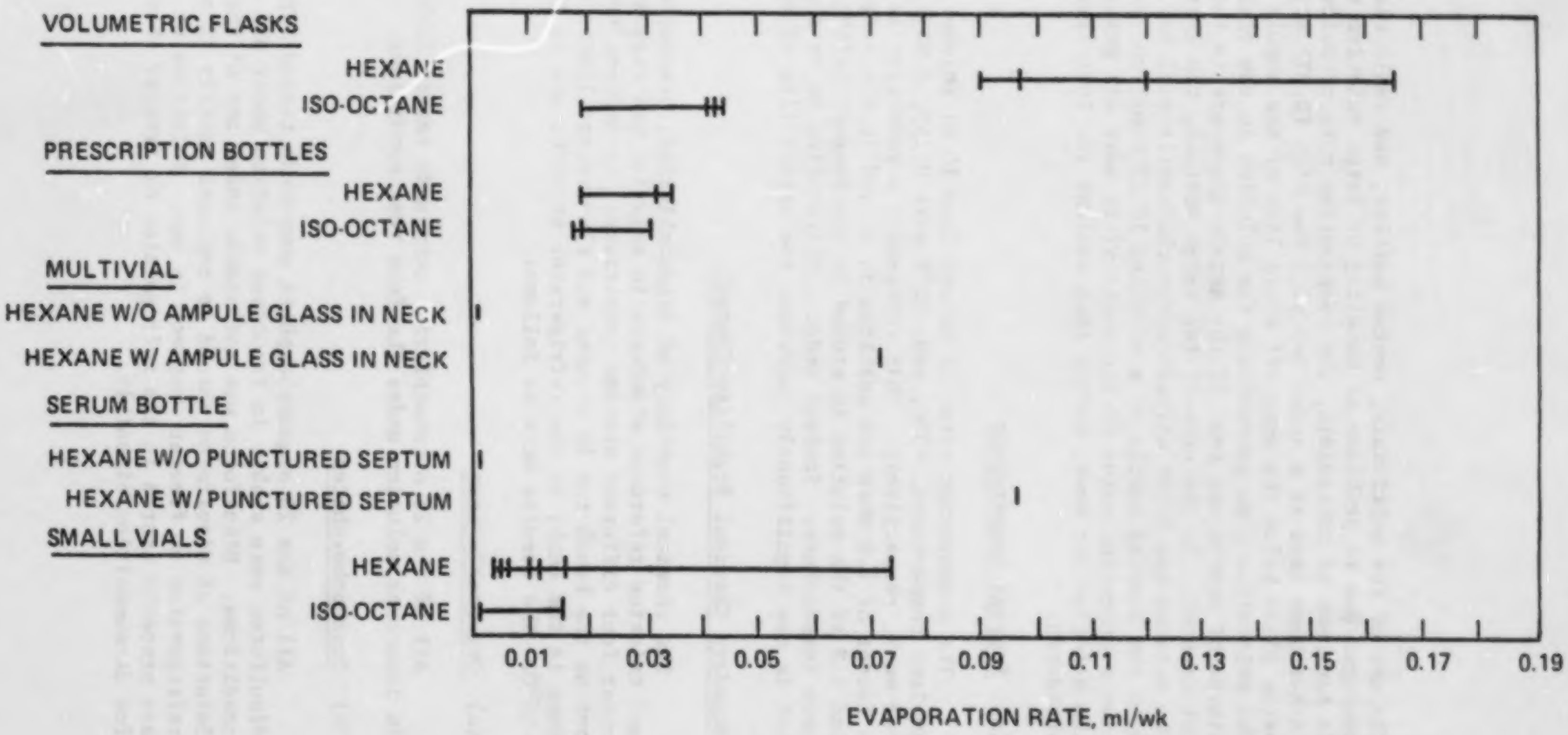


Figure 3-L. Evaporation rates of solvents in different containers



The use of the multivials, septum bottles, and small vials are all discouraged due to problems of handling or large relative evaporation rates. In the case of multivials, the evaporation rate of solvent from the container when used as a screw cap vial was high (0.071 ml/week) unless the extra glass below the snap-off score line of the ampule seal was removed. The multivials also contaminate the solution in the vial with torn up pieces of septum cap seal if the ampule glassware in the vial neck is not removed. In the case of the serum bottles, the evaporation rate of the solvent was 0.094 ml/week after the bottle seal had been punctured with the beveled needle of a standard 10 µl Hamilton syringe. The relative evaporation rates of the small vials were all greater than 5% of the solution per week, making them useless for long-term storage of standards.

(d) Storage temperature

The evaporation rates of hexane from 10 ml volumetric flasks at ambient temperature, +3°C, and -15°C were 0.155, 0.0575, and 0.0226 ml per week, respectively. This represents a reduction in evaporation by a factor of 2.6 when the solution is stored in the refrigerator (+3°C) and 6.8 if the solution is stored in the freezer (-15°C), compared to room temperature. Storage under refrigeration or in the freezer when not in use significantly increases the useful life of standard solutions.

(2) Standard Chemical Stability Control

The chemical stability of organochlorine, organophosphate, carbamate, and triazine reference standards in solution was evaluated for one year under four different storage conditions: at ambient temperature (23-24°C) out on the bench top in natural and fluorescent light; at ambient temperature in the dark; in the refrigerator at +3°C; and in the freezer at -15°C. The results were as follows:

(a) Organochlorines

All of the 28 organochlorine compounds tested (Table 3-6) were stable in isooctane solution under the four test conditions.

(b) Organophosphates

All of the 20 organophosphate compounds tested (Table 3-6) except disulfoton were stable in isooctane solution under all of the four test conditions. Disulfoton was not stable under any of the test conditions. Solutions of disulfoton should be replaced monthly if not stored in the refrigerator or freezer when not in use. Solutions of disulfoton that are properly stored in the refrigerator or freezer should be monitored for decomposition bimonthly.

TABLE 3-6

STABILITY OF PESTICIDE STANDARDS

Test Compounds			
Organochlorines (in iso-octane)	Organophosphates (in iso-octane)	Carbamates (in toluene)	Triazines (in ethyl acetate)
Aldrin	Azinphos methyl	Aminocarb	Ametryn
Aroclor 1016 (tech)	Carbophenothion	Bufencarb	Atraton
Aroclor 1254 (tech)	Chlorpyriphos	Butylate	Atrazine
β-BHC	DEF	Carbaryl	Cyanazine
γ-BHC (Lindane)	Dichlofenthion	Carbofuran	Cyprazine
Chlordane (tech)	Dimethoate	CDEC	Prometon
Chlordene (Kepone) *	Dioxathion	Mecarbam	Prometryn
Chlordene	Disulfoton	Methiocarb	Propazine
DCPA	Ethion	Pirimicarb	Simazine
o,p'-DDT	Ethoprop	Propoxur	Terbutryn
p,p'-DDD	Fenthion	Thiobencarb	
p,p'-DDE	Leptophos		
p,p'-DDT	Malathion		
Dieldrin	Methyl Parathion		
Endosulfan I	Mevinphos		
Endosulfan II	Parathion		
Endrin	Phencapton		
Heptachlor	Phorate		
Heptachlor epoxide	Phosmet		
Hexachlorobenzene	Ronnel		
1-Hydroxychlordene			
Methoxychlor			
Mirex			
t-Nonachlor			
Oxychlordane			
Pentachloronitorbenzene			
Toxaphene (tech)			

*Dissolved in benzene-methanol (99:1 v/v)

(c) Carbamates

Eight of the 13 tested carbamates (Table 3-6) were stable in toluene solution under all four test conditions. Standards of CDEC and butylate decomposed under all four storage conditions. Butylate decomposed approximately 50% per year under all four storage conditions. CDEC decomposed between 50 and 98% per year depending on the storage conditions. Carbaryl, methiocarb, and carbofuran decomposed 38, 48, and 17%, respectively, when stored at ambient temperature with exposure to natural and fluorescent light.

Solutions of CDEC and butylate should be stored in the freezer when not in use and replaced monthly. Solutions of carbaryl, methiocarb, and carbofuran should be stored away from light and replaced after six months.

(d) Triazines

Half of the 10 triazines tested (Table 3-6) were stable to decomposition of the reference standard material in ethyl acetate solution under all four test conditions. Prometryn, prometon, atrazine, and ametryn decomposed between 12 and 17% at ambient temperature with light exposure. Atrazine and atraton degraded approximately 15% at ambient temperature when stored in the dark.

(3) Recommendations for Storage of Pesticide Analytical Reference Standards

The following recommendations are made concerning the factors that affect analytical reference standard solution integrity:

Select a solvent, such as isooctane or toluene, that will dissolve the standard material and evaporate as slowly as possible.

Store the standards in relatively large volume solutions (50-100 ml) to reduce the percentage volume losses to acceptable levels. Monitor the solvent loss by placing an indelible mark on the side of the solution container when the container is filled and then discard the solution when 3-5% of the solvent has evaporated.

Select a container, such as a screw-cap prescription bottle or a large volume volumetric flask, that will not allow rapid solvent evaporation.

Store the standards away from light in a refrigerator when not in use to reduce evaporation and reference material decomposition.

Replace the easily decomposed reference standard solutions at the recommended intervals (e.g., monthly for CDEC, butylate, and disulfoton).

Periodically check standard solutions by comparison against fresh dilutions of the stock solution to prove that the solutions are still valid for qualitative and quantitative use.

Do not store any standard solutions for longer than one year.

3P CALIBRATION AND MAINTENANCE OF THE GAS CHROMATOGRAPH AND ACCESSORIES

It is essential that the entire gas chromatograph be maintained in top operating condition if high quality analytical data are to be produced. In appraising results of interlaboratory check samples, it is clear from data and chromatograms that this is not the case in some laboratories. Section 5 will present details of proper operation of a gas chromatograph. This section will offer guidelines for making routine, periodic checks of equipment to insure continued good operation and minimal down time. Correct procedures for the operations mentioned (e.g., silylation and conditioning of columns, obtaining background profile) will be described in Sections 4, 5, and 6 of this Manual.

Certain checks should be made daily, others on a weekly or monthly basis. Table 3-7 outlines the suggested frequency of such instrumental checks for a chromatograph equipped with an electron capture detector.

It is suggested that a written log be maintained for each instrument, recording the following data:

a. Date of installation and serial number of each detector installed (this will also serve as a record for Atomic Energy Commission inspection).

b. Background current (BGC) profile furnished with the detector under the EPA Interlaboratory Quality Assurance Program or from the commercial manufacturer.

c. Your own BGC profiles obtained at time of installation of each detector and subsequent profiles (column identity notations should be made).

d. Date of change of pyrometer batteries, if used.

A record should also be kept of each GC column packed and installed in an instrument, logging such information as:

a. Assignment of a column number.

b. Date of packing column.

c. Liquid phase identity and lot number of precoated column packing.

d. Conditioning temperature, flow rate, and number of hours.

e. Length and shape of column.

Table 3-7

PERIODIC CHECK LIST FOR GAS CHROMATOGRAPH (EC DETECTOR)

DAILY	WEEKLY	MONTHLY
1. Check response with standard mixture and relate to previous day.	1. Change glass wool plug at column inlet.	1. Clean recorder slide wire with Freon MS-180.
2. Change glass insert sleeve in injection port (end of day).	2. Change septums. 3/	2. Change pyrometer batteries and clean battery contacts with Freon MS-180.
3. Check recorder electrometer zero and noise level at operating attenuation.	3. Run background current profile and polarizing voltage response curve. 4/	3 Check recorder speed at settings in normal use.
4. Check carrier gas flow rate through each column with bubble meter (early morning and afternoon).	4. If endrin is a compound of interest, chromatograph pure standard at a concentration and attenuation that will produce a peak of 50 to 60 percent fsd. 5/	4. Check glass flow system for leaks using "SNOOP". 6/
5. Check temperature of detector, inlet, transfer and column oven. 1/	5. Check for any shifting of column packing resulting in forward movement beyond the bottom of the column exit nut and/or settling in excess of 1/2" from the glass wool inlet plug.	5. Evaluate performance of each column with special standard mixture.
6. Chromatograph standard p,p'-DDT on each working column used at a concentration and attenuation that will produce a peak of 50 to 60 percent full scale deflection (fsd). 2/		6. Check entire instrument for loose connections and frayed wire insulation.
		7. Check all rotameters and flow controllers for proper float action.

1/ Oven temperature should be monitored by an outboard thermometer.

2/ The formation of p,p'-DDD and/or p,p'-DDE indicates on-column breakdown.

3/ This frequency assumes the use of improved silicone rubber. Old type requires more frequent changing.

4/ Daily check may be indicated if large numbers of sample extracts are injected.

5/ The formation of one or two additional peaks indicates on-column breakdown.

6/ Checks should be made whenever a new detector or new tank of gas is installed, or whenever erratic baselines are observed.

f. Background current obtained on newly installed column and subsequent background current profiles during the life of the column.

g. Date of each silylation of column.

h. Compound conversion data, with dates monitored, and percentage of compound breakdown.

i. Monthly, chromatograph the following special column evaluation mixture, recording absolute and relative retention data and efficiency based on the p,p'-DDT peak.

Chlorinated Pesticide Mixture for GC

Column Evaluation

Compound	Concentration, ng/μl
α-BHC	0.010
β-BHC	.040
Lindane	.010
Heptachlor	.010
Aldrin	.020
Hept. Epoxide	.030
p,p'-DDE	.040
Dieldrin	.050
Endrin	.080
o,p'-DDD	.080
p,p'-DDD	.080
o,p'-DDT	.090
p,p'-DDT	.100

3Q ADHERENCE TO OFFICIAL OR STANDARDIZED METHODOLOGY

If reproducible and corresponding data are to be produced on both routine samples and interlaboratory check samples by a group of different laboratories, it is essential that uniform standard analytical methodology be used by all. In the EPA program, this methodology is developed, tested, and collected in the Analytical Manual by the coordinating laboratory of the quality assurance program, in close cooperation with the EPA methods development section. Individual laboratories in the multi-laboratory system are encouraged to suggest improvements in existing procedures, but at no time should any individual summarily introduce method revisions, changes in GC columns, alterations in instrumental parameters, etc., without consultation with the coordinator or authors of methods. Past experience clearly indicates that the vast majority of poor analytical performances on interlaboratory check samples were performed by laboratories deviating in some way from the standard procedures. It is important, therefore, that laboratories adhere to standard analytical methods, but also that they report any problems with them to the coordinator so that these methods can be further researched and improved as experience dictates the necessity. A standard procedure is generally not circulated until such time that reproducibility and precision have been well established. Chemists having troubles with some phase of a standard procedure should search internally for the cause of the difficulty rather than making revisions in the method that cannot be fully studied and statistically evaluated by the individual.

3R IMPLICATIONS OF AN INTRALABORATORY QUALITY CONTROL PROGRAM

An intralaboratory quality control program such as described in the preceding pages requires a good deal of time and effort and does not come cheaply. It is a conservative estimate that around 15% of the typical analytical laboratory's resources should ideally be channelled into quality control. The questions often arise, particularly in a smaller laboratory, "Is such a program worth all this effort and expense? What is the return on the investment?"

Each laboratory administration officer must resolve the answers to these questions in light of the impact of his ultimate analytical data. If his laboratory is regulatory in nature, would he feel comfortable going to court to defend the validity of his analytical data? Would his control program hold up under a barrage of cross-examination questions? If the laboratory's work is primarily of a monitoring nature, would he, for example, feel fully confident in advising his superior officials that a given waterway is carrying a pollution load of x micrograms per liter of PCBs?

From observations in the EPA interlaboratory quality assurance program (Section 2), it can be stated without reservation that laboratories lacking a systematic internal control program more than likely will do very poorly in the analysis of a blind sample. In numerous instances, laboratories joining the program and analyzing a blind for the first time have performed rather badly in contrast to the peer laboratories that have been practicing rigid internal quality control. The practical implication of this, of course, is that analytical data from such loosely controlled laboratories are simply unreliable.

To cite a specific instance, one laboratory joining our program and reporting the results of their first analysis of a spiked water sample reported the presence of p,p'-DDE, p,p'-DDT, o,p'-DDT, heptachlor epoxide, o,p'-DDE, and dieldrin. The actual spiking composition was HCB, oxychlordane, p,p'-DDE, p,p'-DDT, and Aroclor 1254 (PCB). In other words, the analyst found two of the compounds that were actually present, four that were not present, and missed three that were present.

It takes no great stretch of the imagination to assess the reliability of routine analytical data from this laboratory. Such data would do far more harm that good.

Unfortunately, laboratory administrators are sometimes inclined to regard analytical data as a production commodity, expecting x numbers of analyses to be completed in y length of time with little thought to such ancillary factors as quality control or specific analytical problems related to certain samples. We have no great quarrel with output norms, provided that quality control activities are built into the norms. When they are not, analytical data such as those described above should not be regarded as unusual.

3S REFERENCES

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Section 4

EVALUATION, STANDARDIZATION, AND USE OF MATERIALS FOR PESTICIDE RESIDUE ANALYSIS

4A ADSORBENTS

Cleanup and preliminary fractionation of sample extracts are most often accomplished by chromatographic elution through a column of an active adsorbent. Florisil, a synthetic magnesium silicate, is most widely used for this purpose; but the latest trends indicate that partially deactivated silica gel and alumina, charcoal, and adsorbent mixtures, as well as gel permeation chromatography (1), are becoming increasingly popular.

The activity (adsorptive strength) of adsorbents can be checked by elution of standard dye materials (2), lauric acid, or standard pesticide mixtures through a prepared column. Most materials may be activated by strong heating, and some may be activated for a particular purpose by pretreatment with an acid or base (e.g., alumina) or an organic solvent (e.g., charcoal). Deactivation of a polar adsorbent to a desired level has been achieved by addition of a certain percentage of water.

Florisil has proven to be nonuniform in elution characteristics (3,4) and, therefore, each batch requires careful pretesting of the adsorptive properties prior to use. One activity of the EPA Health Effects Research Laboratory, Environmental Toxicology Division, Analytical Chemistry Branch Interlaboratory Quality Control Program (Section 2) is the furnishing of uniform, standard quality Florisil to other EPA laboratories and to laboratories with direct contracts to conduct environmental monitoring. Procedures specified by the program coordinator and other available standardization methods will be described in the following subsections.

a. EPA Procedures for Handling and Evaluation of Florisil

Details are given in Section 3D of the EPA Pesticide Analytical Manual. Especially high quality lots of Florisil (calcined at 1250°C) are purchased from the manufacturer in 200-400 lb quantities after favorable evaluation of an advance sample by the coordinator of the interlaboratory program. When the entire lot is received, another evaluation is made on plugs taken from each polyethylene-lined fiber shipping drum by means of a grain trier. If satisfactory, adsorbent is transferred from the drums to specially cleaned wide mouth glass jars with foil-lined metal screw caps and a capacity for 2 lb of Florisil.

Evaluation of Florisil for use in a modified Mills, Onley, Gaither procedure is made by heating Florisil in an Erlenmeyer flask overnight or longer at 130°C in a clean oven that is preferably dedicated to this sole purpose. Heated Florisil is stored at 130°C in the oven with the flask covered by aluminum foil or glass stoppered. Three columns (Kontes 420530, size 241, 25 mm od x 300 mm length) are packed with 4 inch beds of activated adsorbent topped with Na_2SO_4 immediately prior to use, as described on page 6 of the EPA PAM, Section 5,A,(1),(a). Alternatively, the columns may be prepacked, activated, and stored with aluminum foil covers in the oven, and withdrawn a few minutes prior to use.

Two standard mixtures containing a total of 17 organochlorine and organophosphate insecticides are prepared at levels of 20-250 pg/µl; 5 ml of each is added to separate columns and 5 ml of hexane is added to the third as a control. Elution is carried out with 200 ml of 6% diethyl ether in petroleum ether in two 100 ml portions, and similarly with 15% and finally 50% ether-petroleum ether. The six eluates are concentrated and injected for analysis by gas chromatography with an OV-17/QF-1 column capable of resolving the mixtures of pesticides in the fractions.

The percentage recovery for each compound is calculated from the chromatograms of the eluate increments and the original standard mixture. Results are recorded on the standard form shown as Table 4-1. The Florisil is evaluated on the basis of the elution pattern and recovery of the pesticides of interest. All chlorinated insecticides should be recovered in the range 90-105% with the possible exception of aldrin, for which recoveries may be low. Some organophosphates, such as carbophenothion, may also yield low recoveries. Ethyl ether should contain 2% v/v ethanol as commercially supplied, or if absolute ether is used, exactly 2% v/v ethanol should be added to obtain the correct polarity which results in the compound elution pattern shown in Table 4-1. The effects of the ethanol constituent may be observed in the following Figure 4-A, wherein three identical mixtures of seven compounds were eluted through three separate but identical Florisil columns. Petroleum ether with no ethanol was used in one column, petroleum ether with the correct 2% ethanol in the second column, and petroleum ether with 4% ethanol in the third column.

A copy of the elution pattern is enclosed with each shipment of Florisil to qualified field laboratories, which should attempt to verify the results. Changes in local conditions, such as packing procedures, temperature, and humidity, can affect the amount of adsorbent or the nature (polarity) of the solvent required for proper elution. Although the method outlined evaluates Florisil for use with certain pesticides in a specific procedure, similar methods can be used to pretest different adsorbents for any residue analysis.

Table 4-1

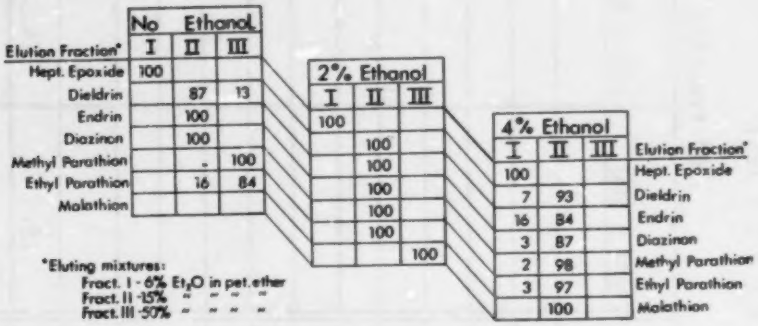
ELUTION PATTERNS AND RECOVERY DATA FOR FLORISIL, LOT #473 ,
BY METHOD SECTION 5,A,(1) (MANUAL OF ANALYTICAL METHODS)
FLORISIL COLUMN PREPACKED AND HELD IN 130°C OVEN AT LEAST 24 HOURS BEFORE USE
RELATIVE HUMIDITY IN LABORATORY 54 %

ELUTION INCREMENTS (ml)

	6% Fraction		15% Fraction		50% Fraction		
Compound	0 - 100	100 - 200	200 - 300	300 - 400	400 - 500	500 - 600	Recovery, %
HCB	100						87.8
Mirex	100						103
Lindane	100						97.4
Heptachlor	100						95.8
Aldrin	100						98.9
Hept. Epox.	57.8	42.2					107
Dieldrin			12.0	88.0			110
Endrin			69.8	30.2			118
p,p'-DDD	100						103
o,p'-DDT	100						103
p,p'-DDT	100						104
Methyl Parathion				100			102
Malathion					75.5	24.5	106
Ethyl Parathion				100			95.6
Diazinon			75.5	24.5			110
Trithion	100						7.7

Numerical values represent the percentage of each compound eluting in the given cut.

Figure 4-A. The effects of polarity variation of eluting solvent in Florisil partitioning of 7 pesticides. Absolute ethyl ether mixed with 0, 2, and 4% absolute ethanol.



	No Ethanol			2% Ethanol			4% Ethanol		
Elution Fraction*	I	II	III	I	II	III	I	II	III
Hept. Epoxide	100			100			100		
Dieldrin		87	13		100		7	93	
Endrin		100			100		16	84	
Diazinon		100			100		3	87	
Methyl Parathion		.	100		100		2	98	
Ethyl Parathion		16	84		100		3	97	
Malathion						100		100	

*Eluting mixtures:
Fract. I - 6% Et_2O in pet. ether
Fract. II - 15% " " " "
Fract. III - 50% " " " "

b. **Florisil Standardization by Lauric Acid Adsorption**

For details, see Section 121.3 of the FDA Pesticide Analytical Manual. Florisil is activated and stored as described in Subsection 4Aa. As an alternative, stoppered containers of activated Florisil may be stored in a desiccator at room temperature and the adsorbent may be reheated at 130°C (unstoppered) after two days.

Standardization by weight adjustment based on adsorption of lauric acid was originally described by Mills (5). An excess of acid solution (400 mg in 20 ml in hexane) is added to 2.00 g of Florisil in a flask, and the amount not adsorbed after shaking for 15 minutes is measured by alkali titration of an aliquot removed from the flask. The weight adsorbed is used to calculate by proportion equivalent quantities of Florisil batches having different adsorptive capacities:

$$\text{Equivalent quantity of Florisil batch required per column} = \frac{110}{\text{Lauric Acid Value of batch}} \times 20\text{ g}$$

$$\begin{aligned}\text{Lauric Acid Value} &= \text{mg lauric acid/g Florisil}\\ &= 200 - (\text{ml required for titration} \times \text{mg lauric acid/ml } 0.05N\text{ NaOH})\end{aligned}$$

This gross method gives no real indication of the elution pattern to be obtained from a column containing the standardized Florisil.

To verify the value obtained by the lauric acid method and to test for proper elution of organochlorine and phosphate insecticides, 1 ml of a standard mixture containing 1-15 µg of eight compounds is applied to a 22 mm id column containing 4 inches of Florisil (or the weight determined by the lauric acid method) and eluted with 200 ml portions of 6, 15, and 50% diethyl ether in petroleum ether. The three fractions are concentrated prior to gas chromatography on an appropriate column. Heptachlor, heptachlor epoxide, ethion, and carbophenothion should elute with good recoveries in the 6% fraction; parathion, dieldrin, and endrin in the 15% fraction; and malathion in the 50% fraction. This mixture is recommended for routine testing since it contains pesticides that give indication of improper elution, poor Florisil, and impure reagents.

c. Deactivated Florisil

Water-deactivated Florisil is required for the Osadchuck _et al_. multiresidue screening procedure for foods (Subsection 9M). Preparation and standardization is carried out as follows for this method (6):

(1) Deactivation

Heat 1-2 kg of Florisil in a one gallon jar at 300°C for 8 hours and cool overnight. Add 2% (w/w) distilled water and place a screw cap lined with aluminum foil on the bottle. Place the jar in a rotary mixer, tumble for 1 hour, and allow Florisil to stand for 24 hours after mixing.

(2) Standardization

A mixture of dieldrin, malathion, and azinphosmethyl is added to a 2.5 cm id tube filled with 15 cm of deactivated adsorbent. The column is eluted successively with 300 ml portions of 30% methylene chloride in hexane, 10% ethyl acetate in hexane, and 30% ethyl acetate in hexane. Dieldrin should elute in the first fraction, malathion in the second, and azinphosmethyl in the third, with all recoveries greater than 90%. Late elution, especially of malathion, which just barely elutes with 10% ethyl acetate, indicates insufficient deactivation and the need for more polar solvents. Early elution indicates over-deactivation, requiring less polar solvents for chromatography (i.e., lower percentage of methylene chloride or ethyl acetate).

Comparable standardization is carried out for other methods employing deactivated Florisil.

d. Deactivated Silica Gel and Alumina

Silica gel deactivated with various percentages of water has been successfully used for cleanup and fractionation in many residue determinations. Preparation of 20% deactivated adsorbent on a small scale has been conveniently and successfully carried out as follows (7):

(1) Activate Woelm silica gel 48 hours at 170-175°C.

(2) Add 2 ml of water to 10 g of adsorbent in a tightly capped Teflon-lined screw top vial.

(3) Mix on a rotary mixer (Roto-Rack™) for 2 hours at setting 8.

Silica gel prepared in this manner can be stored in the capped vial for at least one week with no change in adsorptivity. Standardization is carried out, as above, by packing the required column, adding an aliquot of standardization solution containing the pesticides of interest at a level providing adequate detector response, eluting with appropriate solvents, and examining fractions of eluate by gas chromatography.

Alumina deactivated with water is used in conjunction with silica gel in the Holden and Marsden cleanup procedure (Subsection 9O) and its various modifications (8). This may be prepared in a similar manner by addition of the required percentage of water to alumina previously activated at 800°C for 4 hours.

e. Celite 545

Electron capturing impurities are removed from Celite 545 as follows: Slurry with 6M HCl while heating on a steam bath, wash with water until neutral, wash with several solvents ranging from high to low polarity, and dry. Impurities interfering with phosphorus-selective detectors are removed by heating Celite at 600°C in a muffle furnace for a minimum of 4 hours (FDA PAM, Section 121).

f. Charcoal

Charcoal adsorbent is purified as follows: Slurry 200 g with 500 ml of concentrated HCl, and stir magnetically while boiling for 1 hour. Add 500 ml of water, stir, and boil another 30 minutes. Recover the charcoal by filtering through a Buchner funnel, wash with water until washings are neutral, and dry at 130°C. (FDA PAM, Section 121). As an alternative procedure (9), add 225 g of charcoal to 1.2 liters of ethanol-conc. HCl-water (50:10:40) and reflux for 1 hour. Collect the charcoal on a Buchner funnel and wash with distilled water until pH test paper shows only a trace of acid to be present. Further wash the charcoal with acetone and aspirate until nearly dry. Air dry until odorless (2-3 days) and finally dry in a porcelain dish at 130°C for 48 hours. Store in a tightly stoppered bottle.

g. Magnesium Oxide (Sea Sorb 43)

Slurry 500 g with enough distilled water to cover it in a 1 liter Erlenmeyer flask, heat with occasional shaking for 30 minutes on a steam bath, and filter with suction. Dry for 12-24 hours at 105-130°C and pulverize to pass a No. 60 sieve. About 10% water is adsorbed in this procedure. Store in a closed jar (FDA PAM, Section 121; 9).

h. Packing and Elution of Adsorbent Columns

Pack the adsorbent in glass chromatographic columns containing a loose plug of glass wool (coarse porosity fritted glass discs as support are not recommended because of the difficulty of keeping them clean). Columns 300 mm x 22 mm id with or without a Teflon stopcock (e.g., Kontes 420530, size 241, or equivalent) have been widely used for larger scale cleanup, and 7 mm id columns (e.g., Kontes size 22 Chromaflex columns, or equivalent) for small scale chromatography. Add the required amount of dry column packing in increments and gently tap to settle after each addition; then add a layer of granular sodium sulfate (ca. 0.5 inches) on top of the adsorbent. Prewash the column with hexane or petroleum ether, bring the level of liquid to the top of the bed, add the sample, and wash it into the bed with several small portions of the first eluant. Collect the various fractions in separate containers.

Carry out the elution with a series of solvents and solvent mixtures of increasing polarity. Select the polarity of the solvent series consistent with the activity (polarity) of the adsorbent and the polarity of the sample. Use the least polar solvent that will elute the pesticides from the adsorbent to minimize co-elution of polar impurities.

The order of polarity for several common solvents is as follows:

hexane (petroleum ether) - least polar
benzene
ethyl ether
methylene chloride
ethyl acetate
acetonitrile
methanol - most polar

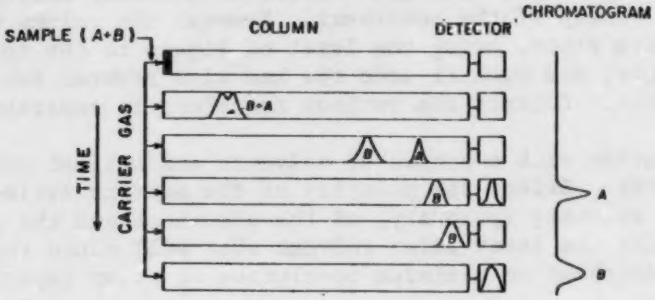
GAS CHROMATOGRAPHY PACKINGS

4B INTRODUCTION AND COLUMN TECHNOLOGY

It is appropriate to reiterate that the column is the "heart of the gas chromatograph." Even though all other modular components of the instrument may be functioning perfectly, a bad column will cause the entire gas chromatographic output to be correspondingly bad. In this subsection, a number of practical operational problems will be discussed; many of these problems have come to light in the interlaboratory quality control program described earlier in Section 2. Some of the operational instructions, fully covered in Sections 4,A- 4,D of the EPA PAM, will be briefly reviewed in this subsection, but only as they relate strongly to the success or failure of the gas chromatographic performance.

A column for gas liquid chromatography consists of a tube filled with a powdered support on which is uniformly coated a liquid, stationary phase. When a mixture of compounds is injected into the gas chromatograph, each compound is swept through the column at a rate that is determined by the interaction of the compound with the stationary phase under the given operating parameters such as temperature and flow rate of carrier gas. If the phase and the parameters are properly chosen, the different compounds will migrate through the column at different rates, and separation will be achieved as diagrammed in Figure 4-B (10).

Figure 4-B. Schematic diagram for elution analysis



Most applications of residue analysis are carried out with packed columns of this type rather than wall coated or support coated capillary (open tubular) columns, although the latter are being used now with greater frequency (see Subsection 4M). GC tubes are usually made of borosilicate glass. Copper and stainless steel are best avoided because both can cause decomposition of compounds unless special precautions are taken.

Commercial solid support materials are usually composed of flux-calcined diatomaceous earth that may be treated by acid- or base-washing or silanization. Firebrick, glass beads, and Teflon are other support possibilities. A good support material should be available in narrow and uniform ranges of particle (mesh) size and have a minimum of active adsorption sites for interaction with injected compounds passing through the column, high surface area per unit volume, good thermal stability, and mechanical strength. Although greatly improved in recent years, various supports and different lots of the same support are not necessarily equal in surface area or inertness. Adsorption or degradation of a pesticide on the support can affect the relative retention time and response of the compound. It is important to select the most inert solid support possible for pesticide analysis, with additional special treatment being desirable for columns used to determine certain sensitive compounds (Sections 4F and 4I). Chromosorb W is among the least active diatomaceous earth supports commercially available. As a general rule, column efficiency increases as the particle size of the support decreases, but a greater carrier gas pressure is required to maintain a given flow through columns with smaller mesh sizes. For most pesticide work, supports with mesh sizes of 80-100 or 100-120 will be satisfactory. The presence of very fine particles, those above the upper limit of each individual mesh range, may cause column inefficiency. If it is likely that particles have been broken during shipment or use, thus increasing active sites and exposing untreated surfaces, check to determine whether the mesh size of the solid support is completely within the expected range.

There are a great number and types of liquid phases commercially available. The choice of liquid phase is usually made on the basis of the polarity of the compounds to be separated. Phases recommended for general use in pesticide analysis are described in Section 5L. Recently, liquid phases have been marketed that are purportedly "equivalent" to previously available phases but with greater thermal stability. It is important to determine whether they provide the same relative retention times.

Important column considerations include efficiency and resolution capability, sensitivity (in relation to the detector), retention, compound elution pattern, stability to heat and injection loading, and freedom from on-column compound decomposition. These will be discussed in light of their effect on day-to-day operation of the column.

4C COLUMN EFFICIENCY AND PEAK RESOLUTION

Figure 4-C shows the equations used for calculating column efficiency (in theoretical plates) and the resolution (R), or degree of separation between peaks, from a chromatogram. A numerical value for efficiency, in itself, is of little practical import. However, efficiency is generally synonymous with peak resolution, and this is of considerable importance to the chromatographer. Figure 4-D, for example, shows superimposed chromatograms of standard chlorinated pesticide mixtures on two separate 6-foot columns of 2% OV-1/3% QF-1, one (A) with very poor efficiency (740 total plates) and the other (B) with high efficiency (4,530 plates). It will be observed that on column B, all seven peaks give baseline separation, whereas on the low efficiency column A, poor separation is evident for four of the peaks.

A column efficiency value of 500 theoretical plates per foot for p,p'-DDT is considered to be of minimal acceptability in terms of the generally expected peak resolution. A 6-foot column of 3,000 plates will usually provide acceptable resolution of mixtures encountered in residue analyses. Since the absolute retention time of the peak used for measurement has an effect on the calculated N, it is necessary to choose a standard peak such as p,p'-DDT for comparison of column efficiency. Column efficiency as measured by this equation is affected by noncolumn factors such as dead-volume in the instrument construction or by any gas leaks.

Figure 4-C. Calculation of column efficiency and resolution

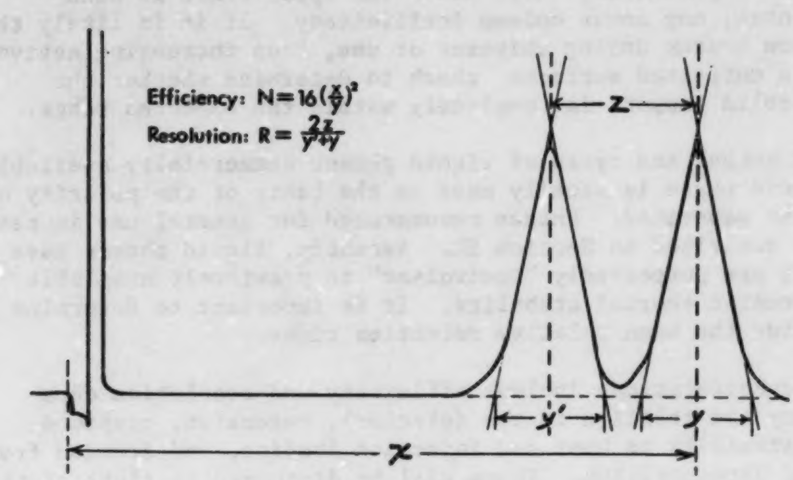
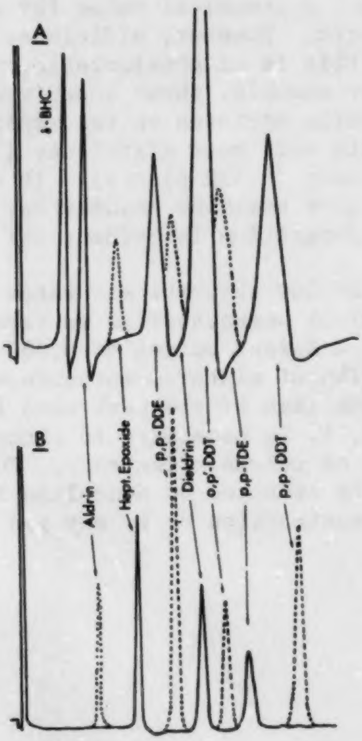

Efficiency: $N=16(\frac{x}{y})^2$
Resolution: $R=\frac{2z}{y'+y}$
z
y'
y
x

Figure 4-D. Effect of column efficiency on pesticide resolution

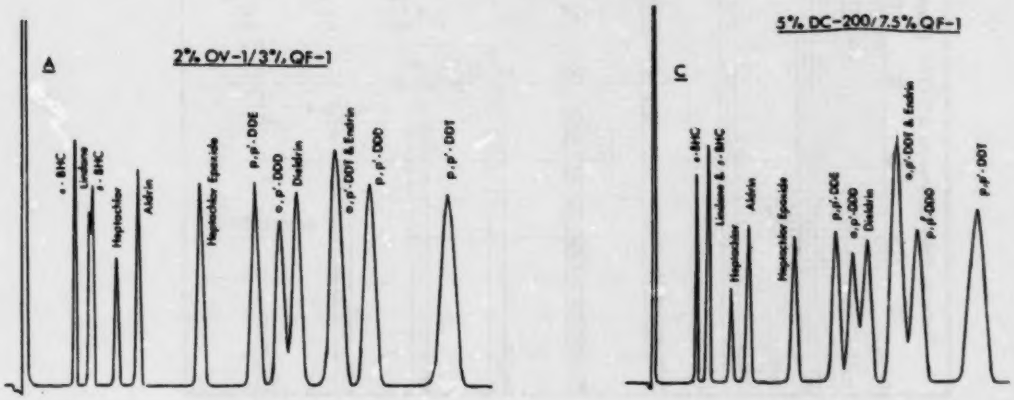


Column factors that influence efficiency are the particle size of the support (small particles lead to higher efficiency), uniform coating, care in handling and packing the coated support, column diameter and length (longer columns provide more total plates), and operating parameters such as temperature and flow rate, particularly the latter. These parameters must be optimized in relation to the liquid phase loading and the analysis time. In general, lower temperatures and flow rates and low liquid phase loading beneficially affect efficiency. Figure 4-E illustrates the advantage of low loading (column A) by comparison of resolution and elution time for two columns of nearly equal polarity operated at similar temperatures. A pitfall of low-loaded columns, however, is easier degradation and/or adsorption of certain susceptible pesticides, affecting both the retention time and the apparent response of these compounds. The minimum coating that can be used is limited to the amount for complete coverage of the support, usually 1-3%, and also by the reduced capacity for sample components.

Figure 4-E. Effect of stationary phase loading on column efficiency.

A: Temp. 187°C, 70 ml/minute, effic. 4550 theor. plates.

B: Temp. 190°C, 100 ml/minute, effic. 2600 theor. plates.



4D SENSITIVITY AND RETENTION

The same principal factors influence the sensitivity and retention of the column: type and loading of the liquid phase, carrier gas flow rate, column temperature, column length, and particle size of the support. These column parameters influence the sensitivity in that any change increasing the peak height for injection of a given amount of pesticide will thereby increase detector response. The columns recommended in this Manual (Subsection 5L) are designed for adequate resolution consistent with practical elution times, and an absolute retention of 16-20 minutes for p,p'-DDT has been found to approximate these characteristics for a column. This retention range can be obtained by operation of lower load columns (3-6%) under such conditions that will produce maximum efficiency. Higher load columns must be operated at elevated temperature and flow rate, and therefore decreased efficiency, to obtain this elution time. Relative retention times are affected only by the nature of the liquid phase and the column temperature. That is, at a constant temperature, the percentage loading of a particular liquid phase can be varied without changing the relative retention of two or more pesticides.

The following bar graph, Figure 4-F, provides comparative sensitivity data on eight GC columns using the 3% OV-1 column as unity for reference purposes. Each column included in the study was operated at its optimum parameters in terms of the achievement of maximum response, efficiency, and a practical retention time.

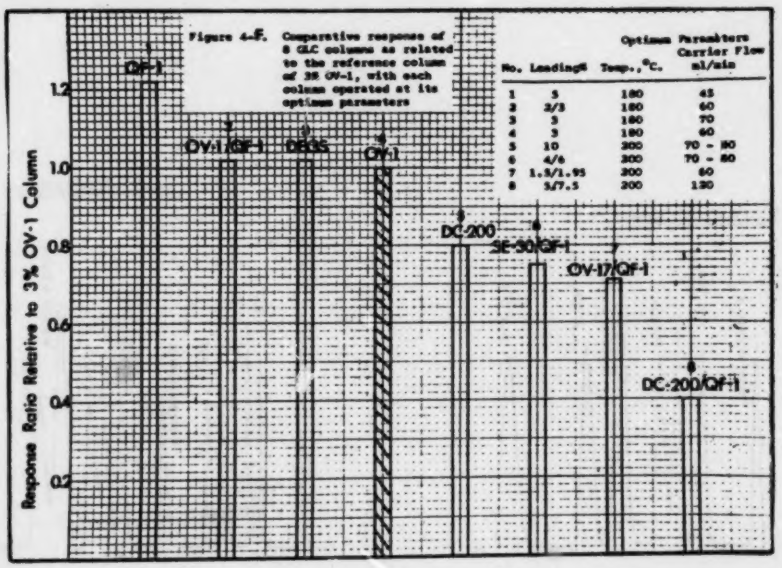


Figure 4-F. Comparative response of 8 GLC columns as related to the reference column of 3% OV-1, with each column operated at its optimum parameters

No.	Loading%	Optimum Parameters Temp., °C.	Carrier Flow ml/min
1	5	180	45
2	2/3	180	60
3	3	180	70
4	3	180	60
5	10	200	70 - 80
6	4/6	200	70 - 80
7	1.5/1.95	200	60
8	5/7.5	200	120

4E COLUMN STABILITY

It is desirable to use columns that are heat-stable or "bleed" resistant and that continue to function properly under injection loading with dirty extract. Liquid phase bleed is evident from a persistently drifting baseline and the inability to obtain a normal level of standing current (Subsection 5C) from an electron capture detector. Minimum baseline noise and drift are achieved with a relatively lightly loaded column containing a stable liquid phase of low volatility.

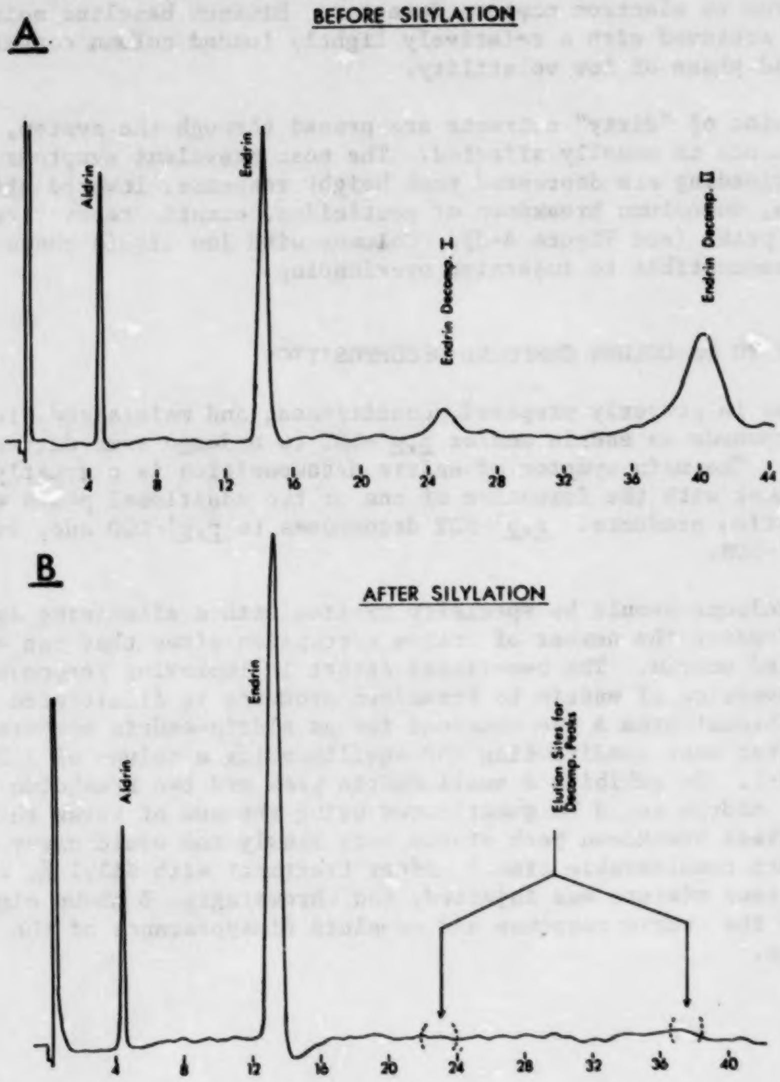
When a succession of "dirty" extracts are passed through the system, the column performance is usually affected. The most prevalent symptoms of injection overloading are depressed peak height response, lowered efficiency and resolution, on-column breakdown of pesticides, erratic recoveries, and unsymmetrical peaks (see Figure 4-J). Columns with low liquid phase loading are more susceptible to injection overloading.

4F RESISTANCE TO ON-COLUMN COMPOUND DECOMPOSITION

Unless a column is properly prepared, conditioned, and maintained, it can cause such compounds as endrin and/or p,p'-DDT to undergo some degree of decomposition. The main symptom of endrin decomposition is a greatly reduced endrin peak with the formation of one or two additional peaks arising from decomposition products. p,p'-DDT decomposes to p,p'-DDD and, in extreme cases, to p,p'-DDE.

Newly packed columns should be specially treated with a silanizing agent such as Silyl 8 to reduce the number of active adsorption sites that can cause decomposition of endrin. The beneficial effect in improving response and minimizing conversion of endrin to breakdown products is illustrated in Figure 4-G. Chromatogram A was obtained for an aldrin-endrin mixture immediately after heat conditioning and equilibrating a column of 1.5% OV-17/1.95% QF-1. It exhibits a small endrin peak and two breakdown peaks. (In principle, endrin could be quantitated using the sum of these three peaks; however, the final breakdown peak elutes very slowly and would cause the analyst to waste considerable time.) After treatment with Silyl 8, the same amount of the same mixture was injected, and Chromatogram B shows significant improvement in the endrin response and complete disappearance of the two breakdown peaks.

Figure 4-G. Reduction in breakdown of endrin resulting from column silanization



Silanization does not always provide such dramatic results. Cases have been noted when no endrin response whatever, either in the form of a main peak or breakdown peaks, was obtained, and silanization did not improve the situation. On the average, however, silanization clearly improves the gas chromatographic behavior of endrin.

DDT breakdown is manifested by the appearance of p,p'-DDD and/or p,p'-DDE on the chromatogram resulting from the injection of pure analytical grade p,p'-DDT that is known to be free of these metabolites as impurities. This problem is associated with overloading of the column packing adjacent to the front glass wool plug, the plug itself, or the glass insert if off-column injection is used, with contaminants from dirty extracts. Figure 4-H illustrates the DDT breakdown phenomenon. Chromatogram A is an aldrin-DDT mixture on an SE-30/QF-1 column with no decomposition, while B shows another column containing the same phase (operated with somewhat different parameters) that caused a total of 25% decomposition of the DDT peak to its two metabolites. This chromatogram was obtained in a laboratory where the injection insert had not been changed for three weeks.

Figure 4-H. Breakdown of p,p'-DDT on 4% SE-30/6% QF-1 column

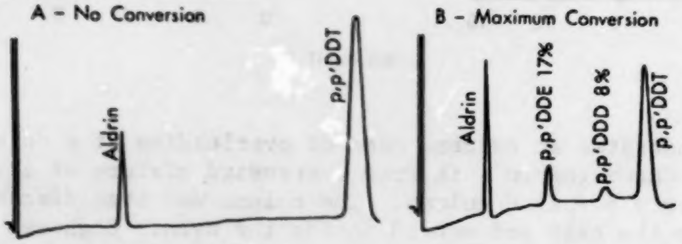


Figure 4-I is a similar illustration of deterioration of column performance with age or with heavy use for dirty samples. The older column (B) is promoting degradation of DDT to DDD (peak 5 to peak 4), and retention times have lengthened. These chromatograms point out the importance of frequent analysis of GC standards that are representative of those compounds that are most frequently analyzed.

Figure 4-I. Electron capture gas chromatograms of DDT and metabolites on a 4% SE-30/6% QF-1 column, 180 cm x 4 mm id, at 180°C. (A) New column, (B) column after 2 months use for "dirty" samples. Compounds: (1) p,p'-DDE; (2) o,p'-DDD; (3) o,p'-DDT; (4) p,p'-DDD; and (5) p,p'-DDT.

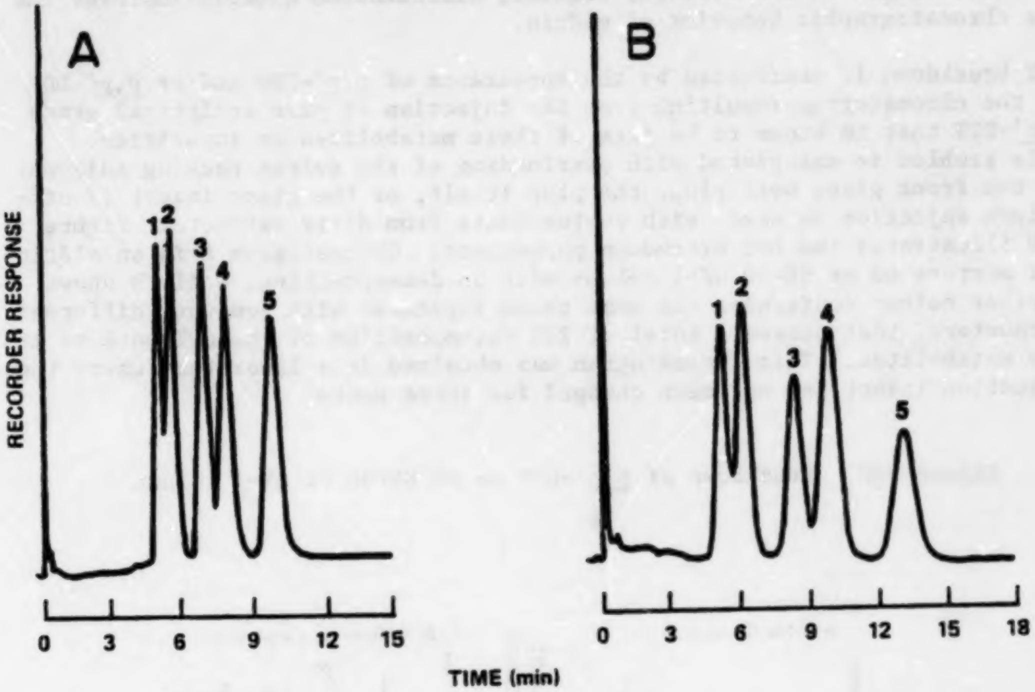
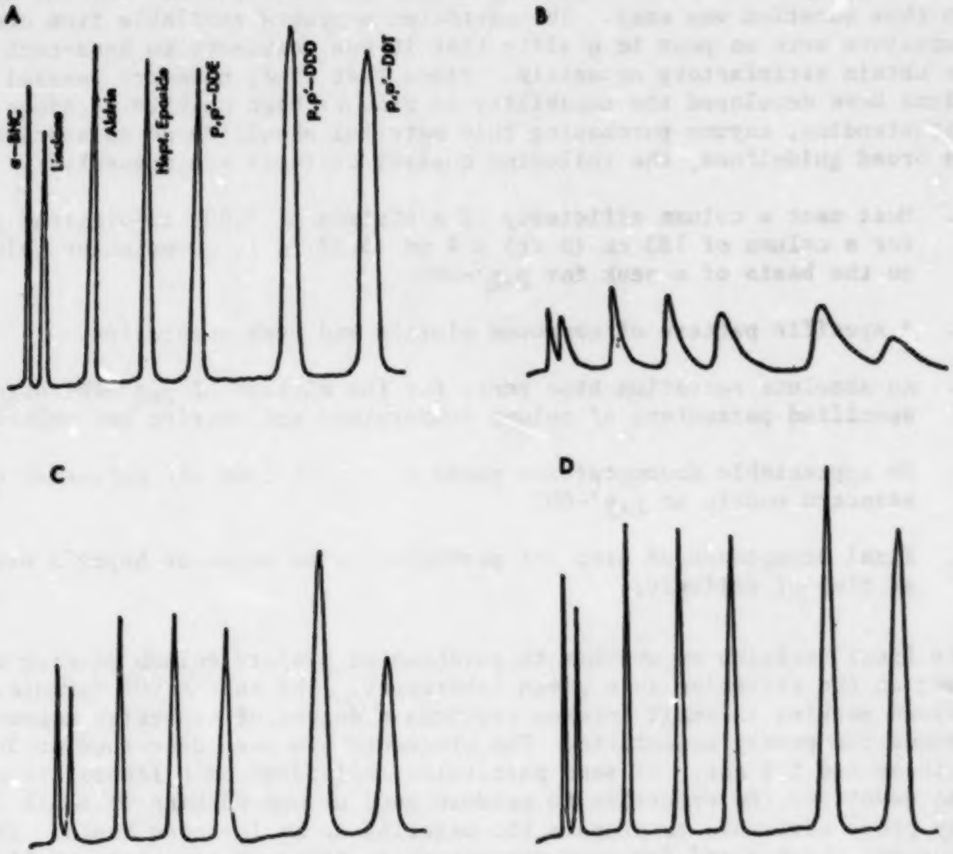


Figure 4-J illustrates an extreme case of overloading of a column of 2% OV-1/3% QF-1. Chromatogram A is from a standard mixture of seven pesticides on a freshly prepared column. The column was then disconnected from the detector so the exit end vented inside the oven. Eighteen consecutive injections were then made of fatty tissue extract after elution with 15% diethyl ether-petroleum ether through a Florisil column, each injection containing the equivalent of 25 mg of fat. After 30 minutes the column was reconnected to the detector, the system was equilibrated, and an identical volume of the same standard mixture was injected. Chromatogram B shows the results of column overloading: depressed peak heights, peak tailing, peak broadening, and conversion of p,p'-DDT to p,p'-DDD (in actuality, the ratio of these changed from 8:10 to 4:10). A clean Vycor glass insert was then installed in the injection port, the system was re-equilibrated for 30 minutes, and another equal volume of standard mixture was injected. Chromatogram C shows the dramatic recovery of the system after this single step. Finally, Chromatogram D indicates a complete rejuvenation of the system when the same mixture was injected after overnight purging at normal operating temperature and carrier flow parameters.

This series of chromatograms is striking evidence that damaged columns can often be salvaged by changing the injection insert, forward glass wool plug, and perhaps the first one-half or one inch of column packing. More importantly, properly maintained and monitored columns should provide top performance without problems for many thousands of injections.

Figure 4-J. Chromatograms illustrating column overloading and subsequent rejuvenation



4G HOMEMADE VS. PRECOATED PACKINGS

The decision whether to make column packings or to buy them precoated confronts every laboratory conducting GC analyses. Prior to 1969 the answer to this question was easy. The precoated supports available from commercial suppliers were so poor in quality that it was necessary to hand-coat packings to obtain satisfactory materials. Since that time, however, several commercial firms have developed the capability to produce high quality packings. Notwithstanding, anyone purchasing this material should do so on specification. As broad guidelines, the following quality criteria are presented:

a. Must meet a column efficiency of a minimum of 3,000 theoretical plates for a column of 183 cm (6 ft) x 4 mm (5/32 in.), computation being made on the basis of a peak for *p,p*'-DDT.

b. A specific pattern of compound elution and peak separation.

c. An absolute retention time range for the elution of *p,p*'-DDT using specified parameters of column temperature and carrier gas velocity.

d. No appreciable decomposition peaks to result from the injection of pure standard endrin or *p,p*'-DDT

e. Final acceptance of each lot purchased to be based on buyer's evaluation at time of delivery.

The final decision on whether to purchase or prepare column packing may depend on the situation in a given laboratory. The successful formulation of column packing in small batches requires a degree of expertise somewhat beyond the purely scientific. The procedure has been described as 50% science and 50% art. If some particular individual on a laboratory staff has developed the expertise to produce good column packing in small lots, it may prove advisable to prepare the material on an in-house basis. This is somewhat cheaper and far more convenient in terms of immediate availability. On the other hand, if no individual on the staff has this "knack" and the laboratory has no appropriate equipment for the task, it may prove advisable to rely on a commercial supplier.

There are a number of methods available for the preparation of column packing. The simplest probably is the "beaker technique" wherein the liquid phase or phase mixture is dissolved in an appropriate solvent in a beaker, the support is added, and the mixture is stirred while evaporating the solvent under a stream of air or nitrogen. The strong disadvantage is that the constant hand stirring tends to fracture the support particles.

An extension of the beaker technique is known as the "filtration technique." The slurry in the beaker comprised of liquid phase, support, and solvent is removed by drawing air through the layer of packing on the filter paper by means of a side arm flask connected to a vacuum source.

The "fluidization technique" is a more sophisticated extension of the beaker technique. The slurry in the beaker is transferred to a fluidizer cylinder (Applied Science Laboratories, Catalog Number 13994) so constructed that a high volume of nitrogen can be blown up through the packing from the bottom of the cylinder, while heat is applied by an element at the base of the cylinder.

In the "rotary vacuum technique" the liquid phase or mixture is dissolved in an appropriate solvent in a small beaker and transferred to a Morton flask (Kontes No. K-295900) with indented sides. The support is added and the flask is placed in a variable heat water bath and connected to a rotary evaporator (Rinco). Mixing and solvent evaporation are carried out by rotating the flask under vacuum with applied heat.

Because no preparation technique is presented in the EPA PAM, one method is offered below for the benefit of laboratories that may like to prepare their own packing. While other methods may be equally satisfactory, the rotary vacuum method as detailed here has proved very satisfactory for the production of small batches of GC column packing. The batch size described will provide enough packing to fill three 183 cm x 4 mm columns.

a. Based on a 21 g total batch size, compute the amount of liquid phase(s) to weigh in 30 ml beaker(s) on an analytical balance.

b. Weigh out liquid phase to two-place accuracy. If making mixed-phase packing, weigh each liquid phase in a separate beaker.

c. With a 25 ml graduated cylinder, transfer 15 ml of the appropriate solvent into each beaker. Stir with a 3 inch glass rod until the liquid phase is completely dissolved.

d. Through a glass funnel, transfer each liquid phase solution into one 300 ml Morton flask. Note: From this point on, all solvent used for rinsing beaker(s) and funnel(s) will be measured so that the final solvent volume in the flask will be just sufficient to produce a slurry of about heavy cream consistency when the support is added. This is a somewhat critical point because too little solvent does not permit adequate mixing for uniform support coating, and too much solvent involves an excessive evaporation time for the solvent. A 10 ml Mohr pipet works nicely for adding and measuring the applied solvent. The beaker(s) should be rinsed with four consecutive applications of 7-9 ml of solvent, the exact amount depending on the appropriate solvent/support ratio.

e. After the liquid phase transfer into the flask, place a powder funnel in the flask and add the support. Note: The amount of support to weigh out for a 21 g batch is the difference, in grams, between the total amount of liquid phase weighed and 21 g. For example, with a 21 g batch of packing of 4% SE-30/6% QF-1:

SE-30 ---------------0.040 x 21.0 = 0.84 g
QF-1 ---------------0.060 x 21.0 = 1.3 g
Total liquid phase: 2.1 g
21.0 - 2.1 = 18.9 g of support

f. Attach the flask to a rotary (Rinco) evaporator.

g. Mix *slowly* for 10 minutes at room temperature with just enough vacuum applied to hold the flask on the evaporator.

h. Advance the hot plate control sufficiently to increase the temperature of water in the beaker to 45°C in ca. 20 minutes. Increase the vacuum slightly at the start of heating and continue increasing, a little at a time. *Notes*: (a) By the time the temperature reaches 45°C, the vacuum should be such that the slurry is at a near-boil. This condition should be maintained throughout, until all visible solvent is removed. (b) After the 10 minute initial mixing period, the flask is rotated *very slowly*. This is a *very critical point*. It is generally not possible to slow the power stat or Variac sufficiently to completely accomplish this, and it is necessary to brake further by hand. This requires *continuous* attention by the operator throughout, really a small time investment in light of the importance of good column packing and the length of time good columns should give service.

i. Advance heat gradually to 55°C, applying as much vacuum as possible just short of flushing liquid solvent out of the flask. Remove all visible solvent at this temperature.

j. Advance heat to produce 65°C, applying all vacuum available and rotating *very slowly* and *intermittently*.

k. When all evident solvent is removed, release the vacuum carefully and shut down the assembly. Transfer the flask of packing to an oven and hold at 130°C at least 2 hours, or overnight.

Alternative pan coating and filtration coating procedures are described in the FDA PAM, Section 301.5.

Once a column is prepared, the actual weight percent loading can be determined, if required, by exhaustive Soxhlet extraction in glass thimbles or standard low temperature or thermal ashing procedures.

4H PACKING THE COLUMN

Columns for pesticide analysis are generally 4-7 feet (120-210 cm) in length and 1/8 or 1/4 inch (0.32 or 0.64 cm) od metal or glass. Aluminum columns have been found suitable for chlorinated pesticides, but glass is usually preferred to prevent degradation often associated with metal columns. U-shaped, 6-foot, glass columns are used in the Tracor MT-200 gas chromatograph that is standard throughout the EPA network of laboratories (Section 5). These are cleaned before packing by scrubbing with soap and water and a pipe cleaner, rinsing with water and acetone or anhydrous methanol, and drawing vacuum to dry. Glass columns should be silanized prior to packing for chromatography of especially labile compounds. See also Subsection 5J for information on silane treatment of glass injection inlets.

There are several methods for packing a column, e.g., hand vibration, mechanical vibration, and vacuum. The method of choice may be dictated by the configuration of the column. Thus, vacuum is about the only method for packing a coiled column. A U-shaped column may be packed by any of the three methods. In general, the aim is to pack the coated support tightly to increase efficiency, with the least amount of particle breakage possible to decrease adsorption/degradation problems. The recommended method is hand vibrating, which has produced columns of consistently high quality.

a. The operator should be sure that the column, if intended as a 6-foot column, is really 6 feet in total length, and not some lesser length. Efficiency and retention time are both reduced in a shorter column. For off-column injection in some chromatographs such as the MT-220, the inlet end of the column should be 1 inch shorter than for on-column injection.

b. On each column leg place a mark at a point on the glass that will be just visible at the Swagelok nut when the column is installed in the oven.

c. Through a glass funnel attached to the column, pour ca. 6 inches of packing into each leg.

d. Repeatedly tap the U-bend of the column on the floor for ca. 30 seconds. Note: The glass is fragile and it is. therefore, advisable to place some type of padding such as a magazine on the floor.

e. Repeat this operation, adding ca. 6 inches at a time to each column leg. It is advisable to vibrate additionally with a wooden pencil, running it up and down the length of the packing.

f. Continue adding packing and vibrating until the pencil marks are reached and the packing will not vibrate below the marks. This should be done with great care, tapping the column a sufficient length of time to be certain that no further settling is possible by manual vibration. The use of mechanical vibration is not advised as the packing may be packed too densely, thus introducing the possibility of excessive pressure drop when carrier gas is applied.

g. Place plugs of ca. 1 inch length of silanized glass wool in each end, just tightly enough to prevent dislodging when carrier gas is applied but not so tight as to impede gas sweep through the column. If glass wool is packed by hand, the hands should be carefully washed with soap or detergent, rinsed, and dried to minimize skin oil contamination of the glass wool. Glass wool can be silanized by treating with 10% dimethyldichlorosilane in toluene for 10 minutes followed by rinsing with toluene and treating for an additional 10 minutes with anhydrous methanol and air drying, or the prepared material can be purchased commercially (e.g., from Applied Science Laboratories). The column is now ready for conditioning.

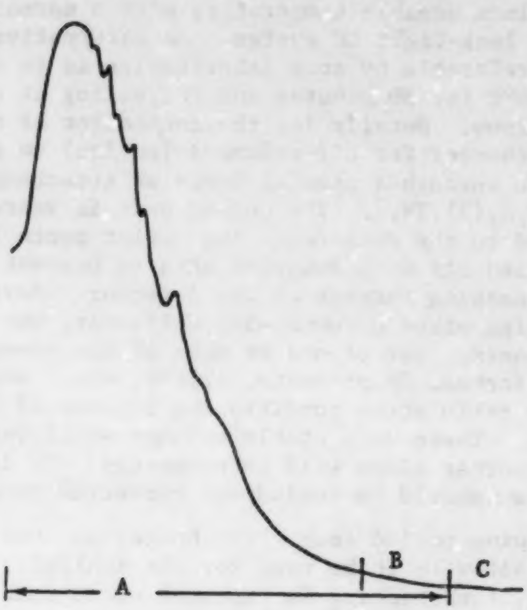
One excellent measure of a well packed column is the net weight of packing per foot compared to a previous efficient column. Experienced chromatographers can repeatedly prepare columns within ca 2 mg/foot using the same batch of packing.

4I COLUMN CONDITIONING

The column is conditioned, or made ready for routine use, by heat curing, silanization, or Carbowax treatment, and injection of a concentrated pesticide solution.

The purpose of heat curing GC columns is to remove impurities in the partition phase, impurities from the solvent in which the phase was dissolved, and the solvent itself. If a column is put into use immediately after coating with these contaminants present, a background signal such as that shown in Figure 4-K caused by the elution of these compounds will result. Proper conditioning will allow the column to operate on the plateau (region C) where a small, constant background signal results from the low vapor pressure of the partitioning liquid at the particular temperature of operation. This low-bleed operation of the column improves day-to-day stability of sensitivity and baselines, quantitation, and the quality of chromatograms; it also lowers the amount of detector cleaning needed.

Figure 4-K. Column conditioning (11).



The following schedule of heat conditioning is recommended for some EPA (Subsection 5L) and FDA prescribed GC columns:

Phase	Oven Temp., °C 1/	Minimum Time, hour
4% SE-30/6% OV-210	245	72
1.5% OV-17/1.95% QF-1	245	48
3% DEGS 2/	235	20 3/
10% OV-210	245	48
10% DC-200 4/	250	16
10% DC-200/15% QF-1 (1:1)	250	72-120
15% QF-1/5% DC-710 (2:1)	240	120

1/ Carrier gas flow 60 to 70 ml/minute.

2/ Shown for information only. Column not recommended for routine use.

3/ Do not exceed this time period.

4/ DC-200 columns are significantly improved if conditioning is carried out without carrier gas (12).

In general, it is desirable to heat cure the column at a temperature ca. 20° below its maximum useable temperature with a normal flow of oxygen-free carrier gas in a leak-tight GC system. An alternative, more gradual approach considered preferable by some laboratories is to use a temperature program starting at 50°C for 30 minutes and increasing at about 5°C/minute up to the desired maximum. Details for the connection of the inlet column leg (which is 1 inch shorter for off-column injection) to the inlet port of a MT-220 chromatograph through a special Swagelok attachment are given in the EPA PAM Section 4,A,(2),IV,1. The column exit is vented inside the oven and not connected to the detector. The outlet ports leading to the transfer line are sealed off with Swagelok nuts to prevent traces of column effluent from seeping through to the detector. Particular caution is needed when preparing mixed columns with different, but supposedly equivalent, liquid phases. Use of one or more of the newer, stabilized liquids (e.g., OV silicones, SP products, silars, etc.) may give a column with an altered phase ratio after conditioning because of increased temperature stability. These more stable columns still require conditioning before use, but shorter times will be necessary. To determine the proper time, the column should be cooled and connected to the detector after a reasonable conditioning period (e.g., 2-3 hours) and the baseline should be checked at the sensitivity to be used for the analysis, or slightly higher. If necessary, conditioning is repeated until stability is satisfactory. Capillary columns, which are also made by evaporation of a solution of a partition liquid, should be conditioned the same way as a packed partition column. The maximum temperature may be lower than for the same liquid in a packed column, however, due to the weaker attraction of the liquid to the column wall.

As mentioned previously, column efficiency and response, especially the response of endrin, would slowly improve as new columns become "seasoned" with use, but silanization is a means of rapidly conditioning the column to full endrin response. After heat curing and with the column still isolated from the detector, the oven temperature and carrier gas flow rate are adjusted to the approximate recommended operating conditions for the column of interest (Subsection 5L). Four consecutive injections of 25 µl each of Silyl 8 (Pierce Chemical Co.) are made, spaced 30 minutes apart. Following the final injection, about 3 hours is allowed for all traces of the silanizing material to elute from the column. The syringe used for these injections should be used for no other purpose and should be rinsed immediately after use to avoid damage. The effects of silanization do not persist indefinitely, and repeat treatment about once a month is recommended. Silanization is particularly useful when low loads of liquid phases are used. Columns to be used with flame photometric or thermionic detectors for detection of organophosphorus pesticides should not be silanized but rather Carbowax-treated.

Generally, Carbowax-treated columns are much more responsive and capable of higher peak resolutions for organophosphate pesticides than columns that are untreated. Depending on the specific compound and column, increases in response have ranged from 10 to 200%, with a 100% increase, or doubled response, being most likely. Silyl 8 conditioning has no beneficial effect on organophosphate response, and silylated columns should definitely not be used with the flame photometric detector since bleed will cause excessive fogging of the heat shield. Details of the treatment and a special Swagelok assembly used in the MT-220 chromatograph are given in Section 4,B,(2),IV of the EPA PAM. This is a modification of the method reported by Ives and Giuffrida to bleed Carbowax from a 2 inch 10% precolumn heated in the chromatograph oven at 230-235°C for 17 hours with a carrier gas flow of 20 ml/minute. (See also Section 4J concerning a different type of Carbowax column treatment.)

The response characteristics of the column should be monitored with a standard mixture of organophosphorus pesticides immediately after treatment to serve as a reference point for later checks on the longevity of the beneficial effects. Response will sometimes drop rapidly for several days after treatment and then stabilize, usually at a level well above that for the untreated column. Carbowax-treated glass wool may also be less adsorptive than the silanized wool usually used.

Following the silanization or Carbowax treatment and with the oven temperature and carrier gas flow rate adjusted to the approximate operating levels for the particular column, several successive injections of a pesticide priming mixture in the microgram range are made onto the column with enough time between injections for all compounds to elute. Injection of priming standards each morning will help assure consistent peak response for working standards throughout the day. With some easily degraded compounds such as underivatized monocrotophos, the column is primed before every analysis. Other difficult pesticides that may not chromatograph well unless the column is aged and primed include perthane, methoxychlor, dicofol, tetradifon, chlorobenzilate, Prolan, captan, esters of 2,4-D, malathion, azinphosmethyl, coumaphos, and PCP.

4J SUPPORT BONDED CARBOWAX 20M COLUMNS

Section 4,A,(7) of the EPA PAM describes the preparation of highly inert GC columns by chemically bonding Carbowax 20M to diatomaceous earth GC support. The Carbowax is coated (using a 5% solution) on acid washed Chromosorb W, 80-100 mesh, and after heat conditioning at 270-280°C, the nonbonded phase is removed by solvent extraction. A thin layer of liquid phase remains unextracted, bonded to the support surface. Columns packed with support prepared in this way, or purchased commercially prepacked under trade names such as Ultrabond (Supelco) or Permabond (Dow), have been used without further treatment or after being conventionally coated with another liquid phase for the separation of chlorinated, phosphate, carbamate, and triazine pesticides and chlorinated phenols.

Support bonded columns have been used with electron capture, Hall electrolytic conductivity, and N-P thermionic detectors (see Sections 4,A,7; 4,C; 4,D; and 12,A of the EPA PAM). The great advantage of these highly deactivated columns appears to be the ability to directly chromatograph polar and unstable compounds without derivatization and to achieve sharp, symmetrical peaks. Support bonded columns allow lower operating temperatures and provide minimal column bleed, longer column stability, and high efficiency and sensitivity (13-16).

Aside from Carbowax 20M, polyester phases have been evaluated for the preparation of support bonded column packings (17). In some cases, double support bonding was advantageous. This involves coating the original heat treated and extracted support with an additional 5% of the same liquid phase and repeating the heat treatment process.

4K EVALUATION OF THE COLUMN

Unfortunately, many chromatographers, after packing and conditioning the column, proceed immediately to use it without making the effort to systematically determine whether it is good or bad. Considering the fact that the column, if properly prepared and maintained, may be in constant use for a year or more as the most vital component of the gas chromatograph, the 2 or 3 hours spent conducting a systematic evaluation is time well invested.

In fact, learning immediately whether the quality characteristics are sufficiently good to justify placing the column on-line as a working tool could result in a considerable overall time saving.

Full details of the evaluation procedure are included in Section 4,A of the EPA PAM. The following material provides highlights of the procedure.

After completion of conditioning steps, the oven and carrier flow are shut down, and the column is connected to the detector. A clean glass injection insert and septum are also installed. The oven temperature and carrier flow are then increased to their operating values. When the proper oven temperature is reached, the carrier flow rate is carefully tested with a soap bubble device and adjusted. (Subsections 5A and 5B discuss the proper performance of temperature and flow rate measurements.) At least 1 hour, or preferably overnight, is allowed for the chromatograph to equilibrate. The temperature and flow rate are rechecked after equilibration. Before making any injections, a background (standing) current profile is run at the normal operating parameters for the specific column being tested if an electron capture detector is used. The polarizing voltage is set at its proper value. These operations are further discussed in Subsection 5C of this Manual.

A complex chlorinated pesticide mixture is now chromatographed to evaluate efficiency, resolution, compound stability, and response characteristics. The mixture described in Section III, C,5 of the EPA PAM is useful for this purpose since it contains compounds that give a number of very closely eluting peaks on the recommended pesticide GC columns. If the mixture is prepared in isooctane and stored tightly stoppered in the deep freeze, it is useable for a year or more for column evaluation (but not quantitation).

From the chromatogram of this mixture, one can calculate the column efficiency based on the peak from p,p'-DDT. For successful pesticide analyses, this should be at least 500 plates per foot, or 3,000 plates for a 183 cm (6 foot) column, as calculated from the equation shown in Figure 4-C. The relative retention time for p,p'-DDT will indicate the actual column temperature (Subsection 5A of this Manual and Section 4,A of the EPA PAM) and serve as a check on the instrument pyrometer readout.

The absolute retention time of the *p,p'*-DDT peak should be 15 to 18 minutes, or the operating parameters are incorrect, the column is not the correct length, or it is not properly packed. Too low an absolute retention indicates too high an oven temperature or carrier gas flow, too short a column, packing which is too loose, or a combination of two or more of these. A high retention time would indicate the possibility of opposite causes.

If column efficiency and resolution are favorable, compound breakdown is evaluated by injection of *p,p'*-DDT and endrin. Columns indicating poor resolution, efficiency, and/or retention characteristics that cannot be corrected by slight parameter adjustments should not be further used. On the other hand, satisfactory columns will often improve or "season" with use, especially as cleaned-up sample extracts are injected onto the column. The percentage composition of the liquid phase undoubtedly changes with age for most columns as well.

Pure analytical standard *p,p'*-DDT and endrin are injected in turn in sufficient concentration to result in a total peak height of 50-60% full scale recorder deflection. Breakdown, as indicated by appearance of peaks in addition to the main pesticide peaks, should not exceed 3% for DDT and 6% for endrin of the amounts injected. The breakdown percentage is the value of all peaks on each chromatogram divided into the total peak area value for the breakdown peaks x 100. Similar procedures are used for other pesticide classes with appropriate standard mixtures.

Reproducibility of the size of peaks when a compound is injected repetitively should be $\lesssim$2-3%. Poor reproducibility can be due to breakdown or adsorption of the compound on the column or to extra-column causes such as faulty syringe or syringe technique (Section 5J), a leaky septum (Section 5J), or detector malfunction. Reproducibility should be checked with those compounds that are possible to chromatograph successfully but that can break down or be adsorbed (e.g., endrin). Priming injections of large amounts of a difficult compound, as mentioned earlier, may allow maintenance of reproducibility for an adequate period of time for an analysis. Difficult compounds should also be checked for linearity of response (Section 50d) since one cause of non-linearity may be on-column breakdown or adsorption.

4L MAINTENANCE AND USE OF GC COLUMNS

Table 3-3 of Section 3 outlines a recommended maintenance program for a gas chromatograph with an electron capture detector in monitoring laboratories in which biological media are predominant samples. A properly cared-for column should provide service for many months. Off-column injection of biological samples will enhance column life (Subsection 5J); frequent (daily) changing of the injection insert and septum helps ensure continuing good performance. Weekly, bi-weekly, or monthly, depending on the number and types of samples injected, the silanized glass wool plug at the column inlet should be replaced. This is mandatory when injecting biological samples directly on-column. If the glass wool plug becomes contaminated by extraneous material, chromatograms showing excessive DDT breakdown, peak tailing, and depressed peak height response will result. Changing the glass wool regularly will usually restore proper performance.

The column packing near the inlet must also be replaced with fresh, conditioned packing if it becomes contaminated. Contaminated packing can be removed without removing the column from the instrument by applying gentle suction through a long-tipped disposable pipet inserted into the column. The column interior should be swabbed where the packing was removed to eliminate fatty deposits on the glass wall. Elimination of the glass wool at the column inlet has been recommended (FDA PAM, Section 301.9) for minimizing fatty extract buildup at the top of the column by permitting the extract to spread over the top portion of adsorbent. This adsorbent, which will trap or degrade pesticides less readily than contaminated glass wool, is regularly replaced. Daily monitoring of DDT breakdown is important for early indication of contamination of the injection port and/or column. Improved cleanup of dirty extracts prior to gas chromatography is an obvious aid in maintaining good column performance.

The effects of silanizing conditioning do not last indefinitely, and breakdown of endrin should be monitored weekly to determine if and when the treatment must be repeated. The effects of Carbowax treatment appear to persist for at least three months under normal use. The operator should watch for a slow decrease in the response of organophosphorus pesticides as compared to that produced by the column immediately after the initial conditioning. A repeat Carbowax treatment of the same column appears to rejuvenate the response, but may cause a shift in some retention values relative to parathion. Repeat treatments are, therefore, not recommended since consistent relative retention values are important for tentative peak identification (Subsection 5N).

When the column is idle overnight or weekends, a low carrier flow of ca. 25/ml minute is maintained through the column and a simultaneous purge flow of 25-30 ml through the detector. When an instrument has multiple columns connected to a single EC detector, a carrier flow just high enough to provide positive pressure is maintained through the unused column(s). In a series of observations with a pair of nearly identical lowload columns having the same 70 ml/minute flow through each, the peak height response for aldrin was reduced ca. 25% compared to when the off-column had a very low carrier flow. If the column not in use is of a highly stable liquid phase such as OV-1, OV-17, etc., the carrier flow on this "off" column may be reduced to zero with no ill effects, thus allowing for full response from the column in use.

Columns removed from an instrument are tightly capped and are reconditioned if out of the instrument for more than a few days. A flow of 60 ml/minute carrier gas for several hours at a temperature ca. 25°C above the prescribed operating temperature (venting into the oven) is used for this operation.

Erratic and noisy baselines frequently indicate leaks in the column connections or some other point in the flow system between the injection port and the detector inlet. If the chromatograph oven can accommodate two or more columns but only one is installed, the unused transfer line to the detector must, of course, be plugged to prevent a massive leak.

Further details of instrument maintenance, troubleshooting, and calibration are given in Section 6.

a. Carrier Gas

Impure carrier gas can often virtually and irreversibly destroy a column. The main manifestations of this are evident in the inability to obtain an adequate background current profile, and low or zero response upon injection of standard solutions. Every effort should be made to avoid installing a new column for evaluation at the same time a new tank of gas is placed on-line. With this situation, the chromatographer cannot be sure whether he simply has a bad column or a bad tank of gas. If the problem is traced to a bad tank of gas, the molecular sieve filter at the inlet of the flow system should also be replaced as experience has indicated that the contamination of the molecular sieve will perpetuate the problem even after a fresh column and good tank of gas are installed (Subsection 5C).

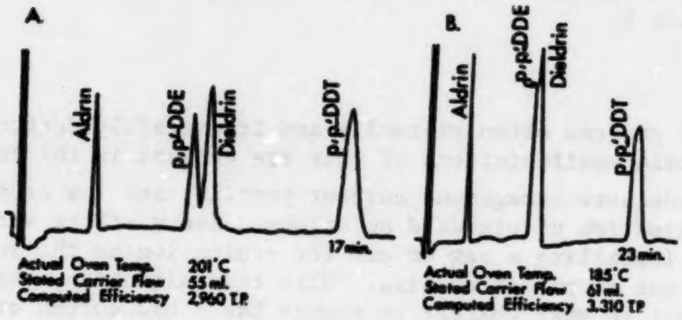
b. Erratic Baselines

This phenomenon may be caused by a number of instrumental factors, and these will be treated in detail in Subsection 5K. The contribution of the column to this problem is largely one of loose joint connections, allowing air to seep into the carrier system. Special care should be taken to ensure that both column joint nuts are tight. One common occurrence is this: The chromatographer connects the freshly conditioned column to the detector and makes certain that the Swagelok nuts are tight. After about two days of operation, the oven door should be opened and the nuts should be tested with a wrench. In almost all cases, it will be found that the nuts are no longer tight, sometimes requiring as much as a half turn for retightening.

c. Accuracy of Oven Temperature and Carrier Gas Flow Velocity

Information gleaned from the interlaboratory check sample program described in Section 2 has clearly indicated that in many laboratories the chromatographer does not really know his true column temperature or carrier gas flow velocity. In most such cases, full reliance is being placed in the accuracy of the instrumental pyrometer and ball rotameter, both of which may be grossly inaccurate. These subjects will be discussed in Subsections 5A and 5B but are highlighted here because of the profound effects on the day-to-day operation of GC columns. Figure 4-L is presented as an illustration. A temperature of 200°C is recommended as optimum for the 1.5% OV-17/1.95% QF-1 column. At this temperature, the separation between p,p'-DDE and dieldrin is normally as shown in Chromatogram A. One laboratory reported operation at 200°C, but their chromatogram was that shown in B. Subsequent investigation revealed that the actual oven temperature was 185°C, or 15°C at variance with the value given by the instrument pyrometer. Resolution or quantitation of either p,p'-DDE or dieldrin would not be possible in Chromatogram B.

Figure 4-L. Effect of temperature on resolution, 1.5% OV-17/1.95% QF-1 column.



d. **Sources of Supply of Blank Glass Columns**

This subject is mentioned here only by reason of a very significant variation in prices between various suppliers for the same commodity. Price markups in excess of 700% are not uncommon, so it behooves the laboratory purchasing group to do a little shopping to achieve the appreciable savings possible on quantity lots.

The cited subsections of Section 5 treat these problems in greater detail as they relate to overall operation of the gas chromatograph.

4M CAPILLARY GC COLUMNS (see also Subsection 5L in Section 5)

The bulk of the material in this chapter concerns traditional packed GC columns, which are predominantly used today in residue analysis. However, applications of capillary GC have increased greatly in recent years.

Coating a capillary column requires the deposition of a uniform 0.1-1.5 μm film of liquid phase onto the walls of the glass tubing, generally 10-60 m x 0.25-0.50 mm id. Coating techniques for wall coated open tubular columns can usually be fitted into one of two general methods, termed dynamic and static. The dynamic method consists of forcing a solution containing approximately 10% liquid phase in a suitable low boiling solvent through the column under closely controlled flow conditions. Usually the coating solution is applied as a single, coherent slug occupying from 2 to 15 coils of the column. The slug is forced through the column at a velocity of ca. 1-2 cm/second with nitrogen pressure. Some workers utilize a single application while others prefer two or three consecutive coating treatments. Several formulas have been proposed for calculating the final thickness of film deposited by the dynamic technique.

In the static technique, the column is completely filled with a dilute solution (3-10 mg/ml) of liquid phase in a low boiling solvent, and one end is carefully sealed. The filled column is placed under vacuum, and solvent is evaporated under quiescent conditions leaving a thin film of liquid phase.

A discussion of these techniques, as well as methods for preparing support coated open tubular (SCOT) and porous layer open tubular (PLOT) columns is contained in the book by Jennings (18). SCOT columns have the liquid phase deposited on a surface covered with a porous layer support material such as diatomaceous earth. PLOT columns have the liquid phase deposited on a surface extended by substances such as fused silica or elongated crystal deposits.

The methods of Grob et al. are probably the most followed by analysts attempting to prepare their own capillary columns. The procedure involves treatment of the glass surface with barium carbonate, deactivation with Carbowax 20M and Emulphor ON 870, and static coating of nonpolar phases and dynamic coating of polar phases (19). The same workers have described a standardized quality test for capillary columns (20).

Onuska and Comba have described the preparation of surface modified wall coated open tubular columns for specific application in pesticide analysis (21). A borosilicate glass column (20 m x 0.24 mm id) was treated with NH_4HF_2 to form filamentary crystals on the inner wall. After heating, the column was washed with 10% HCl, methanol, acetone, and ether, followed by deactivation with a 1% (w/v) solution of Carbowax. The column was then heated to 290°C and dynamically coated using a mercury plug method with a 4% (w/v) solution of OV-101 in n-hexane.

Because of the difficulties in achieving reproducible surface preparation, deactivation, and coating, most workers purchase capillary columns precoated from commercial sources (e.g., Supelco, Applied Science Laboratories). Single phases with a range of polarities are currently supplied. Test chromatograms are usually supplied with the columns, and efficiency is guaranteed at a certain level. A typical value is 2500 plates per meter for a 0.25 mm analytical column.

The stability of capillary columns depends on the liquid phase, the technique of the coating, and the temperature of operation and time of use at that temperature. Some workers have observed that columns last longer if they are maintained at the operating temperature than if they are frequently cooled and heated. The use of dry carrier gas is important, especially when flowing through a cool column. Coated columns store best if they are filled with dry, inert gas and flame sealed. The size and composition of injected samples affect column life. Large injections may have a scrubbing effect that displaces some liquid phase. Some solvents, e.g., CS_2, are especially efficient at displacing liquid phases.

If capillary columns are not used above 260°C, excellent, low dead-volume connections can be made with 30 gauge heat shrinkable Teflon tubing. The glass capillary is carefully butted against the connecting line, and a butane micro torch is used to shrink the covering Teflon tubing and seal the junction.

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Section 5

INSTRUMENTATION AND PROCEDURES FOR GAS CHROMATOGRAPHY

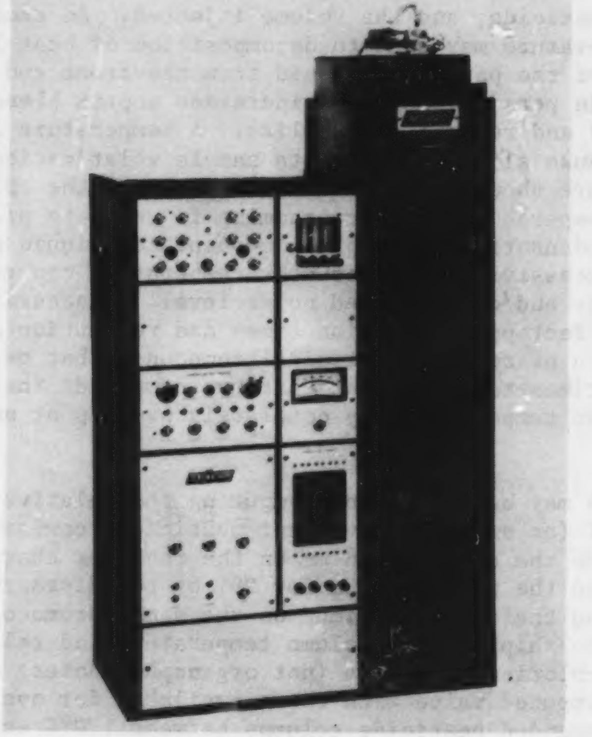
During the extended period of operation of the interlaboratory check sample program described in Section 2, a significant number of analytical "bloopers" have been attributable to improper operation of the gas chromatograph. In many such cases the operator had no idea that anything was wrong, primarily because no systematic guidelines were followed for monitoring the instrumental performance. This section will present such guidelines for the proper operation of the gas chromatograph in pesticide residue analysis. Some of the material repeats the instructions outlined in the EPA Pesticide Analytical Manual, but because of its importance in analytical quality control, it is worthy of reemphasis. Section 4 should be consulted for material on evaluation, standardization, and maintenance of GC columns and Section 6 for details of instrumental troubleshooting and calibration.

Since the gas chromatograph is the instrument in most widespread use in the pesticide residue laboratory, its proper maintenance and use is of primary importance. Failure of any of the components, such as the oven, gas flow system, detector, electrometer, or recorder, to function at optimal potential can markedly distort the overall instrument performance and the resulting qualitative and quantitative data. Table 3-7 in Section 3 outlines a series of periodic checks recommended for insuring a continuing high level of chromatograph performance. Figure 5-A, appearing on the next page shows the MT-220 (Tracor, Inc.) gas chromatograph, which is in widespread use throughout EPA laboratories. This is a floor model chromatograph that features four vertical U-columns, on- or off-column injection, and simultaneous installation of up to four different detectors.

5A TEMPERATURE SELECTION AND CONTROL

Proper adjustment of the column oven temperature and the carrier gas flow rate (Subsection 5B) will have a great influence on the caliber of performance of the entire chromatographic system. Improper selection and control of these parameters may result in poor column efficiency with concurrently poor resolution of peaks, inaccurate relative retention values, depressed peak height response (poor sensitivity), elution times that are either too fast to yield adequate peak resolution and reliable peak identification or too slow to be practical, or rising or erratic baselines. Impaired resolution may preclude accurate quantitation of two important pesticides that are not adequately separated, while inaccurate retention values will make proper residue identification difficult.

Figure 5-A. Gas Chromatograph, Tracor MT-220



The temperature regulation and readout systems of the column oven, detector, and injection port of the gas chromatograph are critical for obtaining reliable analytical results. Accuracy of the pyrometer readouts must be established and maintained to prevent occurrences such as electron capture detector tritium foil vaporization due to excessive temperature or an injection port or column significantly higher or lower in temperature than desired.

A properly operating temperature programmer will maintain the column oven temperature without appreciable deviation (±0.1°C), provided that room temperature fluctuations are minimal. Excessive temperature fluctuation will lead to erratic baselines and retention measurements. Pyrometer batteries (if your instrument is so equipped) should be checked monthly to determine if they are delivering full voltage under load. A hint of inaccurate pyrometer operation is obtained by switching to an unused sensor and observing the readout. A value more than 5°C from room temperature suggests faulty operation. In addition, the oven temperature must be monitored by means other than the built-in instrument pyrometer. A precalibrated pyrometer with leads inserted through the oven door or a mercury thermometer placed down through an unused injection port is recommended. The instrument pyrometer must not be relied upon as the only means of monitoring column temperature.

Injector temperature is determined by the nature of the sample, the identity of the pesticide, and the volume injected. An excessive injection port temperature may lead to decomposition of heat-labile pesticides, stripping of the partition liquid from the front end of the column resulting in peak tailing, and increases septum bleed (leading to spurious peaks) and reduced septum life. A temperature lower than the optimum may cause slow or incomplete sample volatization. The detector temperature should be 30-50°C above that of the column (50°C above the final temperature when programming is used) to prevent the possibility of condensation of sample components or liquid bleed from the column. An excessively high detector temperature can result in reduced sensitivity and/or increased noise level. Inaccurate column temperature can affect peak retention times and resolution and may alter the elution pattern of certain pesticidal compounds that may be present in a sample, sometimes to the extent that two compounds that completely separate at a given temperature may completely overlap at some other temperature.

Column temperature may be checked by computing the relative retention ratio for p,p'-DDT (or another convenient pesticide) compared to aldrin as follows: divide the distance in mm on the recorder chart between the injection point and the peak maximum for DDT by the distance between the injection point and the aldrin maximum on the same chromatogram. There is a linear relationship between column temperature and relative retention values for organochlorine compounds (not organophosphates) so that comparison of this computed value with those available for over 50 pesticides on the recommended pesticide columns between 170°C and 204°C [EPA PAM, Subsection 4,A,(6), Tables 2(a) - 2(c)] should provide a check of the actual column temperature. Selected values for p,p'-DDT are shown in Table 5-1 as an example. A computed relative retention value much below the given value in the table at the selected oven temperature indicates a temperature that is actually higher while a value much higher than the chart denotes a low oven temperature. Relative retention ratios are also a function of the type and proportion of the component liquid phases in the column packing, so preparation of the column and packing should also be carefully checked if retention values are discrepant.

Surveys of the data and chromatograms submitted by laboratories newly participating in the EPA Interlaboratory Control Program (Section 2) indicated that a high proportion of gas chromatographs were operating with column oven temperatures deviating significantly from the supposed

TABLE 5-1

RETENTION TIMES FOR p,p'-DDT RELATIVE TO ALDRIN

Liquid Phase	Temperatures, °C								
	170	174	178	182	186	190	194	198	202
1.5% OV-17/1.95% QF-1	5.57	5.39	5.20	5.01	4.83	4.64	4.46	4.27	4.09
4.0% SE-30/6.0% QF-1	4.04	3.92	3.80	3.67	3.54	3.43	3.30	3.18	3.05
5% OV-210	4.47	4.31	4.15	3.98	3.82	3.66	3.49	3.33	3.17

values. These erroneous temperatures resulted from inaccurate instrument pyrometers and a lack of alternate temperature monitoring devices and procedures. As an example, one laboratory using a column of 1.5% OV-17/1.95% QF-1 indicated an absolute retention time of 26 minutes and a relative retention ratio of 4.87 for p,p'-DDT at a temperature of 200°C and flow rate of 65 ml/minute. Under these stated conditions the retention time for DDT should have been 18-20 minutes, and reference to Table 5-1 shows the true oven temperature was ca 185°C, fifteen degrees less than the pyrometer indicated. See Figure 4-L in Section 4 for illustration of the effects of inaccurate column temperature on peak resolution.

A discussion of the importance of the GC oven to chromatographic performance and suggestions for simple evaluation techniques for thermal variables have been published (1).

5B SELECTION AND CONTROL OF CARRIER GAS FLOW RATE

The exact carrier flow system depends on the chromatograph in use. A common arrangement is for the gas to flow from the cylinder through a two stage regulator to a filter-drier element, branching thereafter to: (a) a purge line running through the purge rotameter, flow controller, and detector, and (b) the carrier gas flow line running through the rotameters, the flow controllers, the column, and finally through the transfer line into the detector. If temperature programming is used, differential flow controllers should be installed in the carrier gas line to prevent a decrease in flow as the pressure drop increases across the column due to increasing temperature.

The choice of carrier gas is dictated mainly by the requirements of the detector being employed. Nitrogen is required for the usual pesticide detectors, except that the pulsed mode of the electron capture detector may employ argon with 5% methane. Flame detectors require gases such as hydrogen, oxygen, and air for combustion. N/P detectors require helium.

Gases should be obtained in the highest possible purity and gas cylinders equipped with dual stage regulators. "Prepurified" nitrogen is required for the DC mode and, in some cases, the constant current pulsed mode of electron capture detection. A gas that is 99.998% pure has an impurity level of 20 ppm, and at least this purity should be employed for the carrier and auxiliary gases in trace analyses. Each gas supply is filtered through a filter-drier cartridge connected at the regulator output of the cylinder. A filter containing Linde 13X (1/16 inch) molecular sieve pellets will remove water, most hydrocarbons, and CO_2. Before the filter-drier is charged with the fresh molecular sieve, the interior of the cartridge is acetone rinsed and heated at 130°C in an oven for at least one hour. The bronze frit is acetone rinsed and flamed. After filling, the unit is heated at 350°C for four hours with a nitrogen flow of ca 90 ml/minute passing through the unit. If activated units are to be stored for a period of time, the ends must be tightly capped. The filter unit should be replaced with a fresh one in the rare event one discovers that a contaminated tank of gas has been used. Oxygen removal requires a special scrubber or a molecular sieve filter immersed in liquid nitrogen. Gas cylinders should always be replaced before they are completely empty.

It is essential that no leaks exist anywhere in the flow system. Even a minute leak will result in erratic baselines with the ^3H or ^{63}Ni electron capture detectors. If the baseline has been stable but becomes erratic upon installation of a new column, a loose column connection is indicated. Leaks are detected by application of "Snoop" or some similar product at all connections in the flow system from the injection port to the detector, provided the connections are at room temperature. Do not attempt to use "Snoop" on a hot column. Commercial high temperature liquid leak detectors are also available for high temperature connections such as around injection ports. These bubble-type leak detectors should be used with caution since the solutions can be drawn into the GC system at the leak source or at a checked source once it is broken.

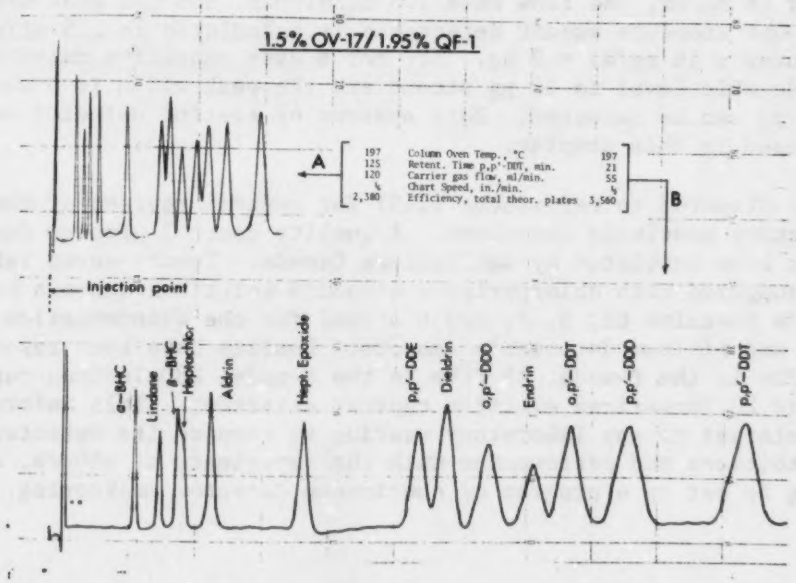
Another means of detecting leaks when using an electron capture detector is by spraying connections with Freon MS-180 with the instrument operating and observing any recorder response. Short sprays are applied close to the connection, but not around the injection port or the detector.

The carrier gas flow velocities are checked using a soap bubble flowmeter, which can be purchased commercially or easily constructed by attaching a sidearm near the bottom of a 50 ml buret [Subsection 4,A, (6), Figure 4(a), EPA PAM]. Since rotameters are installed ahead of the columns, they cannot be relied upon when adjusting the carrier flow as they may be in error. It is necessary to check the flow rate at a point after the column because the pressure drop across columns will vary somewhat from one column to another. An equilibration period of only a few minutes with normal operating parameters is required before the flow rate check is made.

If two or more columns are connected to the same detector via a common transfer line, the carrier flow to the column(s) not in use is shut off while the flow rate through the column in use is being measured. Likewise, the purge gas is shut off. If flow in all columns is shut off, the purge gas flow through the detector can be measured. The flow through unused columns is also shut off while determining the background current of an electron capture detector. The head pressure gauge on some commercial instruments allows continuous monitoring for problems upstream of the column, such as a leak in the carrier gas lines, as well as determination of minimum regulator pressure, changes in column head pressure during temperature programming, and long term column changes. If available, head pressure monitoring can accomplish some of the same results as checking of the flow rate.

Carrier flow rates in excess of recommended values lead to lowered absolute retention times and compressed chromatograms while rates that are too low will have the opposite effect. Relative retention values reflect only the operating temperature of the column (Subsection 5A), while absolute retentions indicate either or both carrier gas flow rate and temperature. Other effects of excessive flow rate may include depression in peak height response and poor column efficiency. Figure 5-B illustrates chromatograms of identical pesticide mixtures from the same OV-17/QF-1 GC column operated with approximately the recommended conditions (B) and then with too rapid a flow rate (A).

Figure 5-B. Effect of flow rate on GC resolution



GC Detectors

GC detectors for residue analysis must be sensitive to minute amounts of the pesticides sought, but selective enough not to detect reasonable amounts of co-extracted substrate material. Despite this selectivity, it is necessary to protect the total gas chromatographic system, including the detector, by purifying the extracts. This will reduce the amount of co-extracted material in the final solution to a level that will not be detrimental to the chromatograph or to the quality of the separation, identification, and measurement. Nonselective detectors, such as the flame ionization detector, produce very complex chromatograms with peaks from pesticides as well as from co-extracted compounds; these detectors are not selective enough to be practical for the quantitative analysis of residues of only a limited number of pesticides of certain classes. With detectors that are less selective (or less specific) for pesticide(s) of interest, more effort is required in sample preparation, in avoiding reagent contamination, and in residue identification.

For pesticide analysis, sensitivity of a GC detector has been traditionally designated as the amount of pesticide that will provide a peak whose height corresponds to some percentage of the full scale recorder deflection (usually 10 or 50%). Minimum detectable amount has been that quantity of pesticide giving a signal at least four times the background noise (random fluctuations) at baseline. Detector sensitivity and minimum detectable level are now generally not differentiated and are reported by instrument manufacturers and many chromatographers in units of weight (μg, ng, pg) per ml for concentration-sensitive detectors (e.g., electron capture GC detector, UV HPLC detector) and in weight per second for mass sensitive detectors (FPD, N-P GC detectors). These numbers designate the concentration or flow rate that will produce a signal level that is some multiple of the noise (usually 2X). If the sensitivity of a compound is stated as 16 ng/ml, the flow rate 1.5 ml/minute, and the peak width 20 seconds, the absolute amount detectable is calculated as 1.5 ml/minute x 20/60 minutes x 16 ng/ml = 8 ng. If, for a mass sensitive detector, the minimum detectable level is 12 pg/second and the peak width is 5 seconds, 12 x 5 = 60 pg can be detected. Both systems of stating detector sensitivity are used in this chapter.

The reader is directed to references (2-5) for general reviews of the element selective pesticide detectors. A quality control program for GC detectors has been initiated by Agriculture Canada. Twenty-seven laboratories were supplied with chlorpyriphos standard solutions (chosen because this pesticide contains Cl, S, P, and N atoms) for the determination of linear range and minimum detectable amounts. Results have been reported (6) for 23 FPDs in the P-mode, 18 FPDs in the S-mode, 28 electron capture detectors, and 20 linearized electron capture detectors. This information will be of interest to any laboratory wanting to compare its detector operating conditions and performance with the experience of others, or those wishing to set up a program of continuous detector monitoring.

5C ELECTRON CAPTURE DETECTOR

a. Principles and Operation

The electron capture (EC or electron affinity) detector is widely used for sensitive detection of halogenated pesticides or other classes of pesticides, often after derivatization with halogen-containing reagents. The detector consists of a radioactive source which emits low energy β-particles (electrons) capable of ionizing the carrier gas to produce secondary electrons. A voltage is applied, causing a steady stream of these secondary electrons to flow from the source (cathode) to a collector (anode) where the amount of generated current is fed to an electrometer and recorded on a recorder. Thus, a standing current or background signal is produced.

When an electronegative species is introduced into the detector, a quantity of electrons will be captured and the current reduced. The negative signal is in contrast to the positive current produced in detectors such as the flame ionization detector. The magnitude of standing current reduction, which depends upon both the number of electron capturing species present and on their electronegativity, is measured on the recorder and indicates the amount of material capturing electrons. After the component passes through the detector, the standing current returns to the original value, and a characteristic GC peak is shown on the recorder, provided that the radioactive foil is not overly contaminated. The exact theory of operation of the EC detector is still unresolved (7-9).

The EC detector is selective in principle for highly electronegative compounds, but in practice it is the least selective of the widely used pesticide detectors. Rigorous cleanup of pesticide extracts is required to eliminate extraneous peaks due to compounds containing halogen, phosphorus, sulfur, nitrogen dioxide, lead, and some hydrocarbons. Its sensitivity, however, is the highest of any contemporary detector, with many halogenated compounds being detectable in low pg (10^{-12}g) amounts. Advantage is taken of this sensitivity by preparing halogenated derivatives of compounds (e.g., carbamate insecticides) normally not detected well by EC. The response of EC detectors has been studied and guidelines presented for predicting which derivatives might best increase sensitivity (10).

Sources of β-radiation have usually been either tritiated titanium on copper or a ^{63}Ni foil. The latter is more expensive but can be used at temperatures above 250°C, which would damage the tritiated detector (maximum temperature ca 225°C). The nickel detector can be used safely to 400°C without appreciable loss of radioactive material. The higher operating temperature reduces the possibility of contaminating the detector with extract impurities or the bleed from GC liquid phases. It also extends the number of compounds that can be detected and greatly reduces detector maintenance. The tritium source is more sensitive than nickel for a short period of time, reaching maximum sensitivity after a few days of operation. Then there is typically a constant loss in sensitivity, requiring frequent recalibration and eventual foil replacement. The sensitivity of the ^{63}Ni cell is reputedly less than that of a tritium cell, but it remains relatively constant and may equal or surpass the sensitivity of a tritium cell after a period of use. Some compounds show increased sensitivity at the higher temperatures possible with the Ni cell than with a new tritium cell (11).

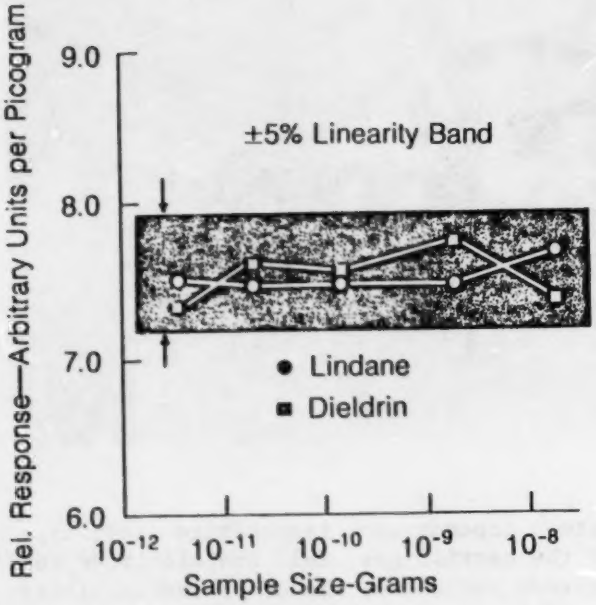
The EC detector is used with either a constant negative DC voltage or an intermittently pulsed DC voltage (constant frequency or "plain pulsed" mode) imposed across the anode-cathode. The former mode requires nitrogen carrier gas, while argon plus 5-10% methane is used with pulsed voltage. The argon-methane can be added to the chromatographic system as a make-up gas or as the carrier gas. When added as a make-up gas, introduced after the column but prior to the ionization portion of the detector cell, nitrogen or helium can be used as the carrier gas, and simultaneous dual detector operation is possible. The pulsed and DC modes provide approximately equal sensitivity and linearity, but advantages have been claimed for the former in terms of freedom from anomalous responses (11), reproducibility of response, independence of response to voltage, and operation with somewhat dirty samples. However, DC operation has proven entirely adequate for routine analyses in the EPA Laboratories when properly cleaned-up samples and low-bleed columns are employed.

Constant current (pulse- or frequency-modulated or variable frequency mode) operation is a third mode of EC detection. A standing current is again achieved by applying voltage pulses, but in this case the pulse sampling frequency is varied by a servo-mechanism closed loop control circuit that maintains the standing current constant even when an electron absorbing compound enters the detector. Pulse frequency is converted to a DC signal that is monitored in the usual way to provide a chromatogram. The basis of quantitative measurement is the relationship between the change of pulse frequency and the concentration of electron capturing substance. This mode of operation provides an increased linear response range without loss of detectability and a high degree of baseline stability (12-14).

Linearized ^{63}Ni constant current EC detectors are available from several commercial sources. They allow detection of low pg amounts of chlorinated insecticides with isothermal or temperature programmed operation and have a linear dynamic range of 10^4-10^5. In one study (15), 27 laboratories reported an average of 1.5 pg of chlorpyriphos required to produce a readily discernable peak. The compound-independent, extended linearity is of great benefit for automated analyses where a wide concentration range of samples can be analyzed without dilutions or reruns. Those detectors with small cell volumes (ca 0.3 ml) are well suited to capillary column GC. Most commercial linearized constant current EC cells can be operated with either argon-methane or nitrogen carrier gas; the linear range may be one decade higher with the former (15). Detector sources are either ^{63}Ni or tritiated scandium. The latter has been found to have a significantly greater linear range and similar sensitivity (16).

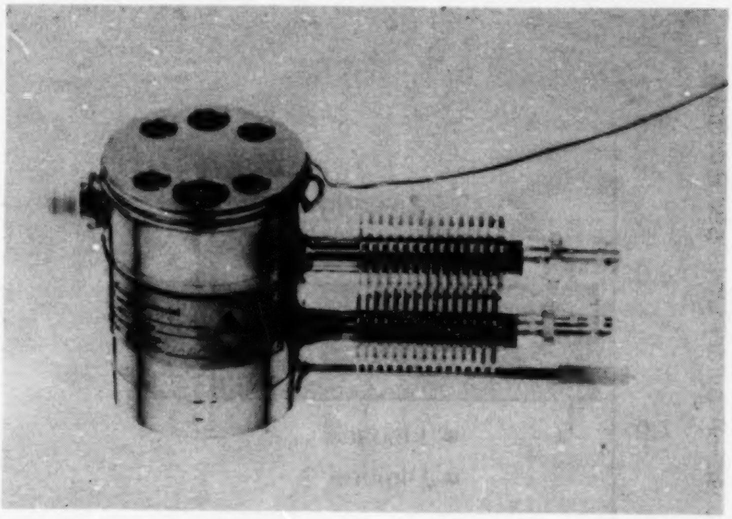
Figure 5-C illustrates the linearity of the Tracor ^{63}Ni electron capture detector equipped with an electronic linearizer (frequency modulated mode) [EPA PAM, Section 4,A,(3),IV]. This detector offers a linearity range of 10^4 with either nitrogen or argon-methane carrier gas. By comparison, the linearity of the Tracor detector in the DC-pulse mode is 10^2-10^3 (17).

Figure 5-C. Response of the Tracor linearized EC detector from 5 x 10^{-12} to 5 x 10^{-8} grams using argon-5% methane carrier gas.



The EPA analytical laboratories originally used parallel-plate ^{3}H EC detectors because cleaning can be done in-house under an NRC permit. Details are given in the FDA Pesticide Analytical Manual, Vol. I, Section 311.12, for cleaning a tritium EC detector. Foils may be removed only by persons with an NRC license for this purpose. A cleaning solution of 5% KOH in absolute ethanol is recommended for cleaning radioactive foils. Tritium foils should not be exposed for more than one hour, and aqueous solutions or traces of water should be avoided. Mildly abrasive cleaning compounds and ultrasonic cleaning apparatus may also be used. High temperature ^{63}Ni detectors are probably in greatest present use, with and without electronic linearizers, and concentric-design tritium detectors are also still widely used. Figure 5-D shows a Tracor, Inc. ^{63}Ni detector. The column effluent entrance is shown on the left and the purge gas line, polarizing voltage connector, electrometer input connector, and gas effluent outlet (top to bottom) on the right. The heater-limit switch fits into the nearest large hole seen on the top, front. The ^{63}Ni foil is a sealed source and is usually sent back to the manufacturer for cleaning. It is frequently possible, however, to clean a nickel foil in the chromatograph by injecting 100 µl of water a few times into a 300°C system employing an empty column. Purging the detector at 400°C overnight may also be helpful. It has been reported (7) that the major contamination of the EC detector occurs by deposition of material on the anode surface, causing a significant reduction in efficiency of collection of electrical charge, and that performance can be restored by cleaning only the anode without disturbing the other parts of the cell.

Figure 5-D. Tracor, Inc. ^{63}Ni EC Detector

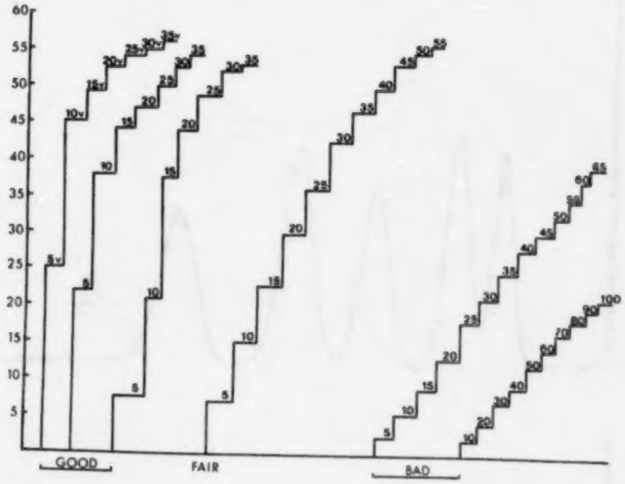


Response of EC detectors depends upon temperature (18); type, flow rate, and pressure (19) of the carrier gas; cell and electrode configuration and dimensions; electrode positions; amount of radioactivity;.contact potentials caused by adsorption of sample components on electrode surfaces; space charges of slow moving ions surrounding the electrodes; and applied potential. The adverse effects of even slight scoring on the EC collector probe have been described (20). The unpredictable nature of these parameters causes anomalous responses, drifting baselines, variable sensitivity, and a limited, variable dynamic range in the DC mode. Operating parameters must be optimized for each manufacturer's detector.

b. Background Current Profile.

Measurement of the background current profile (recorder response vs. voltage) should be made regularly to evaluate the performance of the detector as influenced by the condition of the foil or other factors such as column bleed or contaminated carrier gas. At maximum voltage and an attenuation setting of 12.8 x 10^{-9} A.F.S. when using a 1 mv recorder, a good detector should produce a response of 60-80% full scale deflection. With aging this will approach 30%, when the foil should be replaced. A profile that drops drastically in a period of one or a few days indicates problems with the detector itself or an adverse influence by the column. Detailed instructions are given in Section 4,A,(3) of the EPA PAM for obtaining a BGC profile with a Tracor MT-220 chromatograph, and typical profiles are illustrated in Figure 5-E. Operational details for obtaining background current vary somewhat from one instrument to another, and each particular instrument manual should be consulted for recommended column parameters for making this test. Some commercial detectors regrettably do not provide for easy variation and readout of the potential. In general, more significant information is obtained by determining the background current at the normal operating parameters for the column being used.

Figure 5-E. Typical electron capture detector background current profiles.



From the background current profile, the optimum polarizing voltage for operation may be estimated. If the detector foil is new and the background current is high, it is usually acceptable practice to simply set the polarizing voltage at such a value to give 85% of the total profile with the ^3H detector or at 92% with the ^{63}Ni detector. If operation above this range is attempted, anomalous results can occur. Figure 5-F shows an obvious case in which anomalous results were produced by operating above 99% of the maximum current with a DC mode tritium detector.

Figure 5-G shows a less obvious situation. The upper chromatogram in this figure shows optimum response; the lower one shows abnormal response due to operation at over-voltage. Expansion of the upper portions of the peaks and contraction of the peak bases result, so that, in effect, only the tops of the peaks are seen, as if the broken line in chromatogram A were the baseline. Detector over-current can result from cleansing of the detector foil by heating or injection solvents, and the analyst may be unaware that it has occurred. The problem may also exist when the detector is operated in the AC or linearized modes, as the standing current must be correct to provide a working linear range (see below).

A more reliable method, especially for older, partially dirty detectors, is to run a polarizing voltage/response curve as described in Section 4,A,(3) of the EPA PAM. Selection of the proper polarizing voltage is very important so as to (a) produce maximum peak height (response) with

Figure 5-F. Normal electron capture response (A) to chlorinated pesticide mixture and response (B) resulting from operating at an excessively high applied voltage.

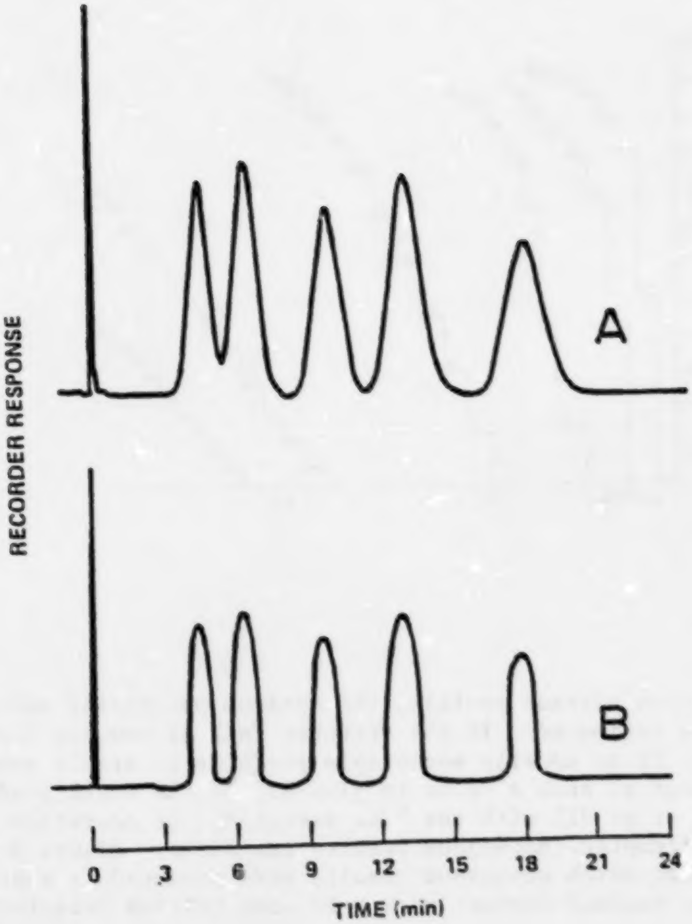
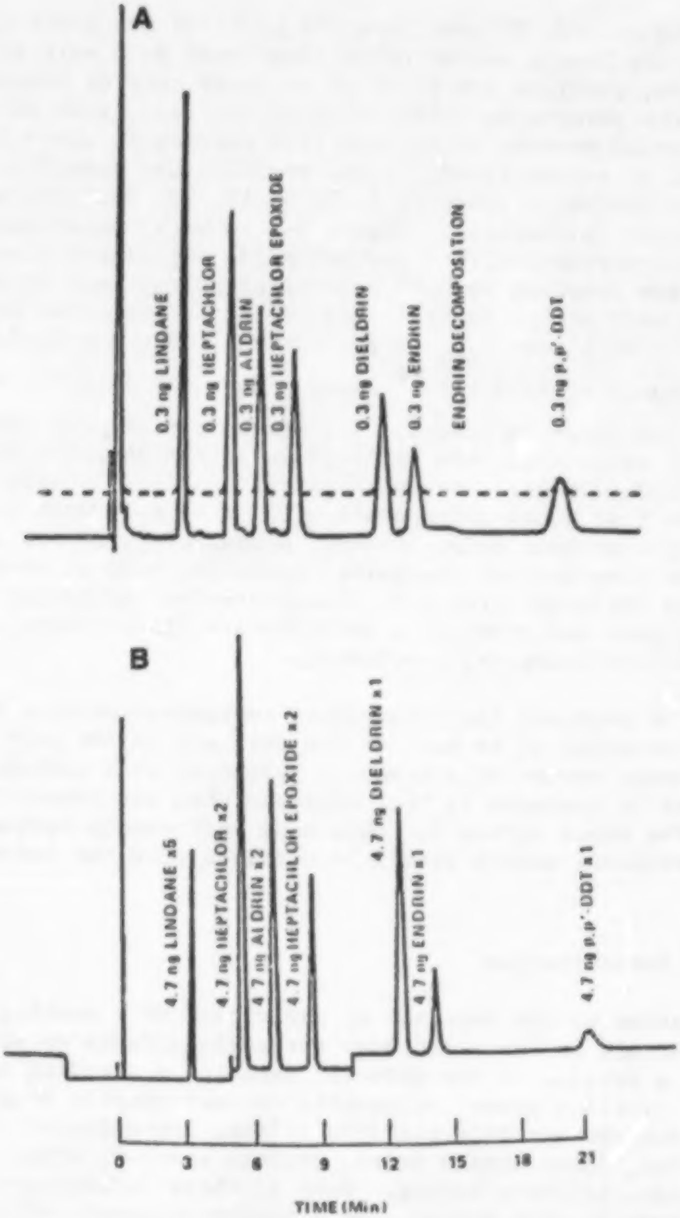


Figure 5-G. Chromatogram of standard chlorinated pesticide mixture. Column: 1.8 m x 4 mm id glass packed with 10% DC-200 on a silane-treated support. Column temperature: 200°C. Detector: electron capture at 1×10^{-8} AFS. (A) detector voltage 10V, (B) detector voltage 30V. Broken line on chromatogram A indicates apparent baseline for chromatogram B. (Courtesy of Applied Science Laboratories, Inc.)



minimum electrical overshoot on the backside of the peak (Figure 5-A,H), and (b) ensure maximum possible efficiency and peak resolution. The polarizing voltage must be adjusted to accommodate a slowly deteriorating background current, so frequent profiles must be run to keep a check on this. An article published in Gas-Chrom® Newsletter, March/April 1973 treated this subject so well that a reprint is presented on the following two pages, courtesy of Applied Science Laboratories, State College, PA.

As shown in Figure 5-E, DC mode detector profiles are plots of detector current along the Y-axis versus polarizing level in 5 volt steps. In the pulsed mode, profiles are plots of response current versus the frequency of the polarizing pulse at baseline, i.e., with no electron capturing material present other than that over which there is no control, such as column or septum bleed. Pulse profiles are normally generated using variable frequency steps of 1, 2, 5, 10, 20, 50, 100, and 200 thousand cycles per second (kilohertz). Figure 5-H shows a comparison of profiles made with argon-methane carrier gas and pulse polarization and profiles made on the same detector with DC polarizing voltage and nitrogen carrier. The left hand pair show a typical clean detector installed on a gas chromatograph. Note that I_{SAT} (argon-methane) is 6.4×10^{-9} amps versus an I_{SAT} (nitrogen) of 4.75×10^{-9} amps. The ratio is 1.35, which will usually hold for clean detectors. The second set of profiles of a very dirty detector shows that this ratio fails if the detector is really dirty, as this one obviously is. The third set of profiles indicates a clearly dirty detector that would not operate well in DC mode with nitrogen. With the pulsed argon-methane mode, however, a completely normal profile is produced. The advantage of the pulsed operating mode is obvious. The ratio is quite different from 1.3. This detector was tested after these profiles were made and produced a satisfactory linear curve of response in the pulsed mode using argon-methane.

A simple way to evaluate the progressive contamination of a detector in pulsed-mode operation is to monitor the decrease in the peak response level for a given amount of a standard pesticide at a certain pulse voltage. This is compared to that obtained when the detector was first installed. The exact method for obtaining performance curves for different pulsed mode detector models should be obtained from the individual operation manuals.

c. Detector Contamination

Contamination of the detector by deposition of a coating of low vapor pressure materials on the electrodes seriously affects detector performance by consuming a portion of the detector capability, leading to loss of sensitivity, trailing peaks, or erratic chromatographic baselines. Contaminant sources may be a bleeding column, contaminated carrier gas, bleeding septum, dirty sample inlet, unclean samples, dirty carrier gas flow controller, or dirty tubing. Many of these factors are treated in detail elsewhere in this Manual, but a review is presented below.

Chromatographer, Beware of Thy Detector!

We all know that the performance of the same types of GC columns can vary with the quality of the packings and the columns themselves. Figures 1 and 2 are examples of extreme differences in column performance when used for pesticide analysis. Both columns are 6 ft x 4 mm ID glass U-tubes packed with 10 wt % DC-200 on a silane treated support. Both runs were made at the same operating conditions. Resolution in Figure 1 is good, but the resolution in Figure 2 is far superior because of the unbelievable 13,000 theoretical plates obtained. Yet, the separation factors are the same in both cases (e.g., 1.12 for endrin/dieldrin).

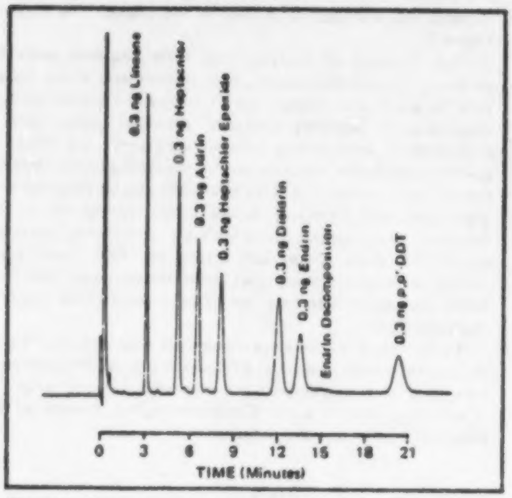


Figure 1. Chromatogram of standard chlorinated pesticide mixture. Column: 6 ft x 4 mm ID glass packed with 10% DC 200 on a silane-treated support. Column temperature: 200°C. Detector: Electron capture at 1 x 10^{-9} AFS.

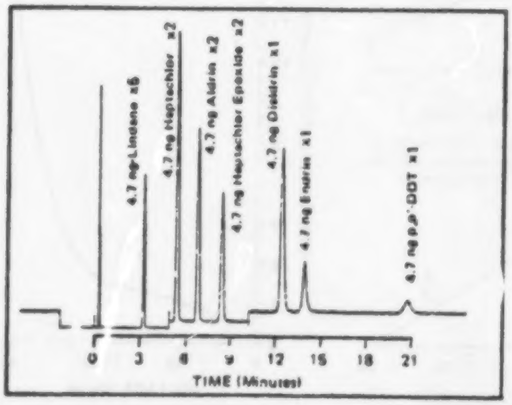


Figure 2. Chromatogram of standard chlorinated pesticide mixture. Column: 6 ft x 4 mm ID glass packed with 10% DC 200 on a silane-treated support. Column temperature: 200°C. Detector: Electron capture at 1 x 10^{-9} AFS.

You say that you would like a 6 ft column with the efficiency shown in Figure 2? Well, we can make such columns and we can obtain results like those in Figure 2 anytime we want to – providing we use an EC detector. In fact, we make these columns all the time, but do not obtain the Figure 2 results – because we do not want to. You ask why? A very good question.

Let's compare Figures 1 and 2 more closely. There is a noticeable difference in absolute detector response between the two results and also in relative responses among the peaks. In Figure 1, the endrin/dieldrin peak height ratio is good (0.61), with signs of slight endrin decomposition, which is normal. However, in Figure 2 the endrin/dieldrin ratio is only 0.31, indicating appreciably greater endrin decomposition, and yet there are no signs of it in the chromatogram. Something appears to be radically wrong with the results in Figure 2.

Well, something is wrong. The column used in Figure 2 does not produce 13,000 theoretical plates. Actually, it is the exact same column which gave the Figure 1 results. Everything was the same for the two runs except for one thing – the EC detector voltages were different. A voltage of 10 volts produced Figure 1, while a voltage of 30 volts produced Figure 2. The detector is an old Barber-Colman tritium EC detector with variable DC voltage; i.e., any voltage can be applied. A plot of background current (baseline) vs. detector voltage is shown in Figure 3. The problem is non-linear detector response. EC detector response is linear or approaches linearity only over a small range of voltages. This voltage range usually lies below the knee leading to the plateau in the current vs. voltage curve (see Figure 3). In our examples, this voltage range is approximately 10 to 15 volts.

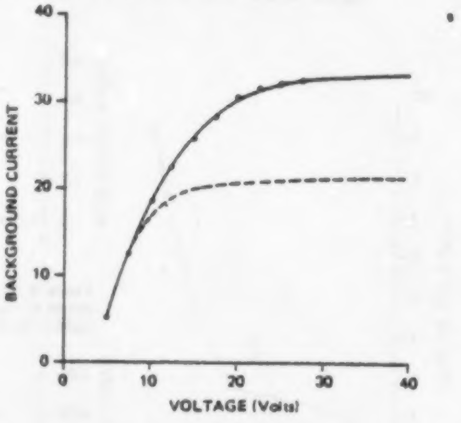


Figure 3. Plot of current vs. voltage for an EC detector.

At higher voltages, the response becomes non-linear and the response-to-concentration slope increases with increasing concentration. This non-linearity becomes extreme on the plateau of the current vs. voltage curve. Here, the response to concentration slope is very small at low concentrations and increases rapidly at high concentrations. This results in an extreme contraction of the lower part of a GC peak and an extension of the upper part of the peak. When this occurs, a chromatogram like the one in Figure 2 is produced. Figure 1 can be converted to an approximation of Figure 2, as shown in Figure 4. A superficial baseline has been drawn which cuts out the bottom part of the peaks. The similarity between Figures 2 and 4 is obvious. If we extended the upper part of the peaks in Figure 4, the chromatogram would resemble that in Figure 2 still more closely. This may appear extreme, but notice that in Figure 2 we have lost not only the endrin decomposition product, but also all the small impurity peaks that are seen in Figure 1.

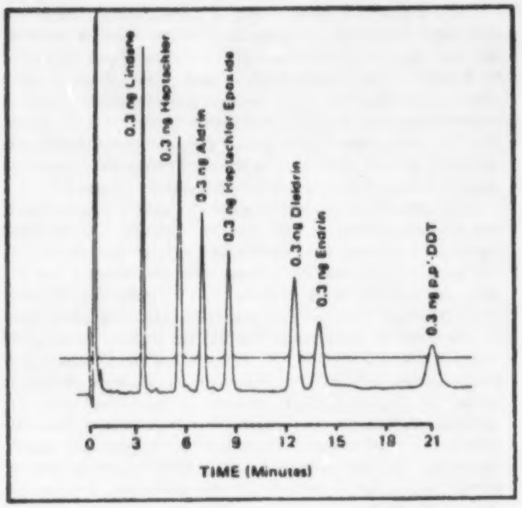


Figure 4. Same chromatogram as in Figure 1.

At voltages below the optimum range, the reverse occurs. The response to concentration slope is high at low concentrations and decreases with increasing concentration. In this case, the lower part of a peak will be extended and the upper part contracted. This is observed as a wider peak, giving a low theoretical plate calculation, and the peak maximum will tend to be rounded. Also, small peaks will be overemphasized.

The graphs in Figures 5 to 8 summarize the effect of EC detector voltage on GC results. These figures show the effect of voltage on various peak height ratios and on the theoretical plate calculation.

Another complication is that the current vs. voltage curve and the optimum voltage are not always the same, but vary with factors such as detector cleanliness and liquid phase bleed. A dirty detector or a high liquid phase bleed will cause the plateau in the current vs. voltage curve to shift to lower currents and voltages, as shown by the broken line curve in Figure 3.

This problem of variable non-linear response with EC detectors complicates quantitative analysis and is the reason why frequent and careful use of calibration standards is so important in pesticide analyses. However, when one is interested in determining column efficiency, the effect of non-linear detector response on the theoretical plate response has not been so obvious. This effect can also be observed with argon ionization detectors, where applied voltage also affects linearity. Years ago we found we were consistently obtaining about 150 more theoretical plates per foot from argon ionization detectors at voltages above the optimum than from flame ionization detectors, which have good linear response characteristics.

To be fair to EC detectors, there are now one or more on the market which operate at a fixed voltage and are claimed to have good linear characteristics over a wide dynamic range.

Let it suffice to say: "Chromatographer, beware of thy detector! Also, know thy detector!"

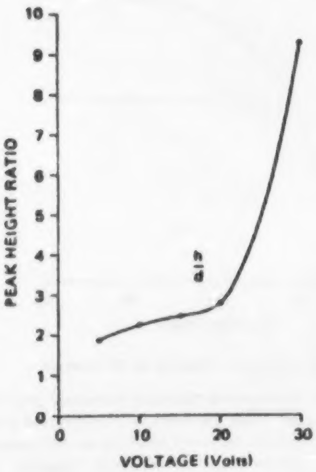


Figure 5. Plot of peak height ratio of heptachlor to dieldrin (h/d) vs. EC detector voltage.

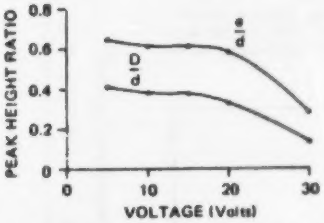


Figure 6. Plot of peak height ratios of endrin to dieldrin (e/d) and p,p'-DDT to dieldrin (D/d) vs. EC detector voltage.

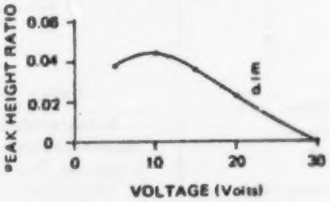


Figure 7. Plot of peak height ratio of endrin decompostion product to dieldrin (E/d) vs. EC detector voltage.

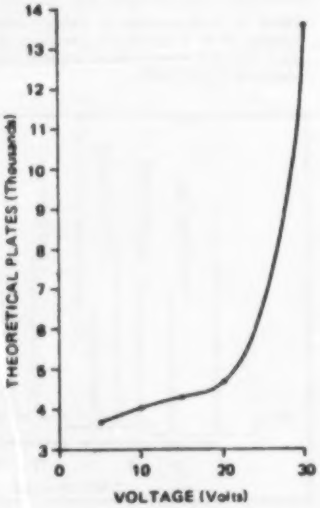
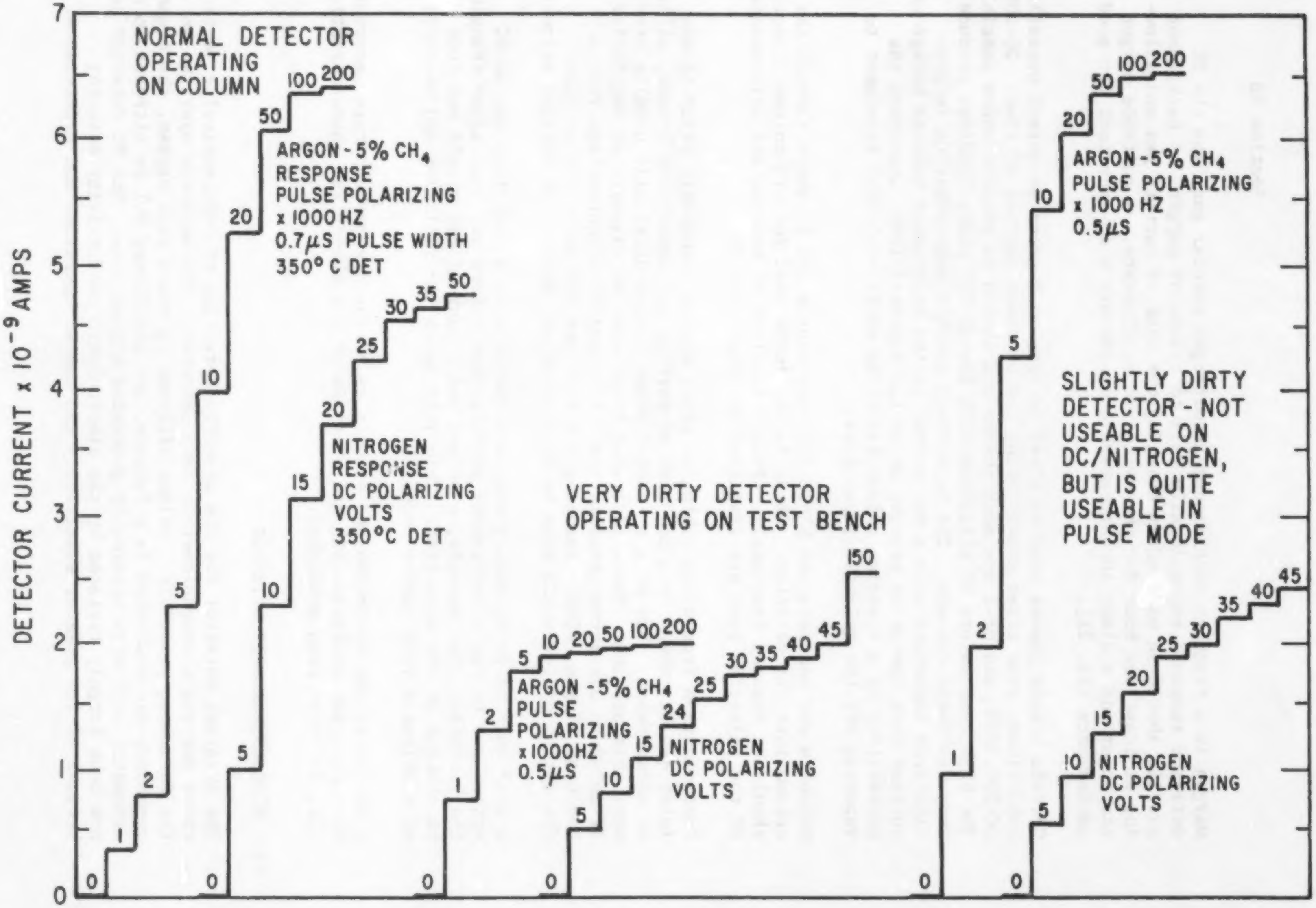


Figure 8. Plot of calculated theoretical plates for dieldrin vs. EC detector voltage.

Figure 5-H. Pulsed and DC mode electron capture detector profile comparison.



Oxygen is a frequent contaminant in nitrogen carrier gas, and the EC detector responds exceptionally well to traces of oxygen. A background profile should be made after changing the tank of carrier gas and allowing at least one hour for the system to equilibrate. A suitable oxygen scavenger and a clean chromatographic system are most important for good performance (13, 21).

Certain liquid phases tend to bleed in varying degrees at normal operating conditions, even after conditioning for extended periods of time. DC-200, DC-550, DEGS, and QF-1 are such phases and should be avoided where possible. The high temperature OV silicones with low (1-5%) phase loadings produce very favorable columns. The background current determination is particularly important with a new column in the instrument because background current that cannot be brought up to the expected level indicates the probability of a bleeding column requiring additional heat treatment to vaporize off the volatile impurities.

Solvents and monomers can bleed from the septum and be swept through the column into the detector. Glass inlet liners used for off-column injection should be changed frequently. Proper handling of septums and maintenance of the injection port are discussed in Subsection 5J.

Contamination from dirty tubing or other system components prior to the inlet can be caused by a bad tank of carrier gas containing grease, oils, or water vapor. Use of a molecular sieve filter-drier will usually prevent this problem. These adsorbent traps must be regenerated regularly. If moisture has accumulated in the tubing and flow controllers from a bad tank of gas, simply changing the tank may not solve the problem. The entire system would have to be flushed out with a low boiling solvent.

A good rule is to introduce only one variable at a time into the GC-EC system and to run a background profile just before and just after changing the variable. For example, a column and a tank of gas should not both be changed at the same time. This rule makes the isolation and correction of problems a much easier task.

A review of the operation and principles of the electron capture detector for pesticide analysis (22) and a review of its theory and characteristics (11, 23) have been published.

5D MICROCOULOMETRIC DETECTOR

The original detector for the specific detection of organochlorine pesticides was the microcoulometric (MC) detector. The detector operates on the following principle: column effluent is mixed with oxygen, the organic compounds are combusted in a furnace, and the formed HCl is titrated in an automatic cell with internally generated silver ions. The MC detector has now been largely replaced by the electrolytic conductivity detector because of its greater sensitivity and easier operation and maintenance.

The MC detector can also be operated to be specific for pesticides containing S, P, or N, but the FPD, N-P, and electrolytic conductivity detectors are preferred for the detection of these compounds. One advantage of the detector for some applications is that the amount of ions used in the cell reaction can be related theoretically by Coulomb's law to the absolute amounts of pesticides passing through the GC column. Although the detector can still be purchased from Dohrmann-Envirotech and is occasionally reported in the literature (24), the absence of wide use in EPA laboratories and pesticide analytical laboratories in general has dictated that detailed material on this detector be deleted. Interested readers are referred to earlier revisions of this Manual and the FDA PAM (25).

5E THERMIONIC DETECTORS

The original alkali flame ionization detector was described by Karmen and Giuffrida in the early 1960's. They found that if a crystal of an alkali salt is placed over a flame and a collector electrode above the alkali source, a very enhanced response to phosphorus-containing species could be obtained. A variety of designs of the alkali flame ionization detector (AFID) were reported, the mechanism of operation was widely studied, and further modifications made the detector sensitive to nitrogen as well as phosphorus compounds. The AFID is described in detail in the FDA PAM, Section 313 and in Section 5E of earlier revisions of this QC Manual and is reviewed in references (26) and (27). The alkali flame, Coulson, and flame photometric detectors have been compared and evaluated (28).

New developments in the 1970's, pioneered mostly by Kolb et al. (29), have led to a thermionic detector involving an electrically heated bead, resulting in more reliability and an extended linear range. This detector, termed the nitrogen-phosphorus or N-P detector, is described in Section 4,D of the EPA PAM and Section 316 of the FDA PAM and will be discussed in the rest of this section. N-P detectors are supplied by different manufacturers (see below). A schematic diagram of the Perkin-Elmer detector incorporating a rubidium silicate bead is shown in Figure 1 of Section 4,D of the EPA PAM. In the usual mode of this detector, shown in the center of this Figure 1, the source is electrically heated, and the detector is sensitive to both nitrogen- and phosphorus-containing substances, but more sensitive to P than to N. The linear range is over five orders of magnitude, and sensitivity is in the pg range. In the P-mode, shown on the right of Figure 1, the source is heated by a high energy flame and the jet is grounded. The response to phosphorus is the same as in the N-P mode, whereas the N response is suppressed ca 50 fold and the linear range is reduced to 10^4.

Compared to the KCl AFID, the N-P detector operates with reduced flow of hydrogen gas and temperature. This causes an increased response to nitrogen while maintaining high sensitivity to phosphorus.

The principle of operation of the N-P detector is as follows: GC column effluent is ionized at or near the electrically heated alkali source in the presence of a relatively low temperature plasma around the bead. P- and N-containing species are preferentially ionized and drawn to the collector electrode, where the resulting change in current is amplified and recorded.

For detection of N-containing pesticides, the N-P detector is less selective than the N-mode of the Hall electrolytic conductivity detector. However, the former is more stable, sensitive, and easier to operate. Sample extracts can often be examined after a minimum of cleanup using the N-P detector (see the FDA PAM, Sections 232.4 and 242). Extracts containing traces of acetonitrile cause a large detector response that obscures early-eluting pesticide peaks. Since the usual mode of the N-P detector detects phosphorus and nitrogen compounds simultaneously, a single extract can be conveniently examined for both types of compounds. For examination of P compounds only, the P-mode of the FPD is the usual best choice. Some models of the detector do not tolerate injection of halogenated solvents without deterioration of the alkali source. This should be determined prior to use.

N-P detectors are available from Perkin-Elmer, Hewlett-Packard, Varian, and Tracor. Although each is basically a flame ionization detector to which an electrically heated source has been added between the jet and ion collector, they differ in design and operation and in the exact nature of the alkali source (see the EPA PAM, Section 4,D,II). Installation, operation, and maintenance instructions should be carefully followed for each of the detectors. The detectors include a power supply for heating the source and for maintaining source bias voltage. An electrometer as used for an FID is required. High purity hydrogen and air should be used for the detector, and nitrogen or helium for the carrier gas.

The following are general characteristics of the various N-P detectors: selectivity to nitrogen against both hydrocarbon and phosphorus increases with decreasing hydrogen flow, but selectivity to P vs hydrocarbon is not greatly influenced by flow rate. The electrical potential difference between the jet and collector can also affect selectivity, but this effect varies with individual detector designs. Selectivity factors range from 10,000-100,000:1 for N vs C and 75,000-200,000:1 for P vs C. Under detector conditions providing a 1/2 fsd response for 2 ng of parathion, μg amounts of hydrocarbons or S- and halogen-containing compounds should cause no response. The N/P response ratio is 10-20:1.

Sensitivity is dependent upon the temperature of the source, which is a function of the source heating current and the flows of air and carrier gas, and to the flow of hydrogen gas. Manufacturer's optimal specifications are ca 10^{-13} grams N/second and 10^{-14} grams P/second. Greenhalgh and Cochrane (17) reported a minimum detection level of 0.12 or 0.13 pg chlorpyriphos for three different N-P detectors, which represented a ten fold better sensitivity than the FPD (P-mode). The FDA PAM reports practical detection limits of 1 ng for most OP pesticides and 5-30 ng of most nitrogen pesticides. Sensitivity for nitrogen pesticides varies with the number of N atoms in the molecule and its structure. Compounds containing a P=O group are about twice as well detected as the analog with a P=S group (17). Detector linearity is at least three orders of magnitude and as high as five orders.

It has been noted by analysts that differences among alkali sources are common, even as supplied by one manufacturer. Recommended gas flows are: air - 30-200 ml/minute, with 100 ml/minute being usual; less than 10 ml/minute is required if He rather than N_2 is the carrier gas; hydrogen - 1-5 ml/minute, usually ca 3 ml/minute; carrier gas - 15-30 ml/minute. Sensitivity is lower at carrier flows above 40 ml/minute. To accommodate this lower flow rate, 2 mm id packed columns rather than 4 mm id columns can be used. Similar chromatograms are produced in a 2 mm id column with a 15 ml/minute flow and in a 4 mm id column at 60 ml/minute, with a constant operating temperature.

The body of the detector is normally at 250°C and the source at 700-900°C. The temperature is chosen by adjusting the potentiometer to produce a baseline current as recommended by the manufacturer, usually 1.5×10^{-11} amps or 4×10^{-12} amps. Or, the temperature can be set to produce a specific response for a standard amount of pesticide, e.g., 1/2 fsd for 2 ng of parathion.

To reduce contamination of the detector, low bleed septa should be employed, columns should be properly conditioned, and injection of "dirty" samples should be avoided.

Figure 5-I shows a photograph of the Tracor N-P detector, and Figure 5-J is a schematic of the mounting detail for connection to a gas chromatograph. Figure 5-K shows a typical chromatogram using this detector. The selectivity for the N- and P-containing pesticides compared to eicosane (C_{20}) is obvious.

Figure 5-I. Tracor Model 702 N-P detector and control module

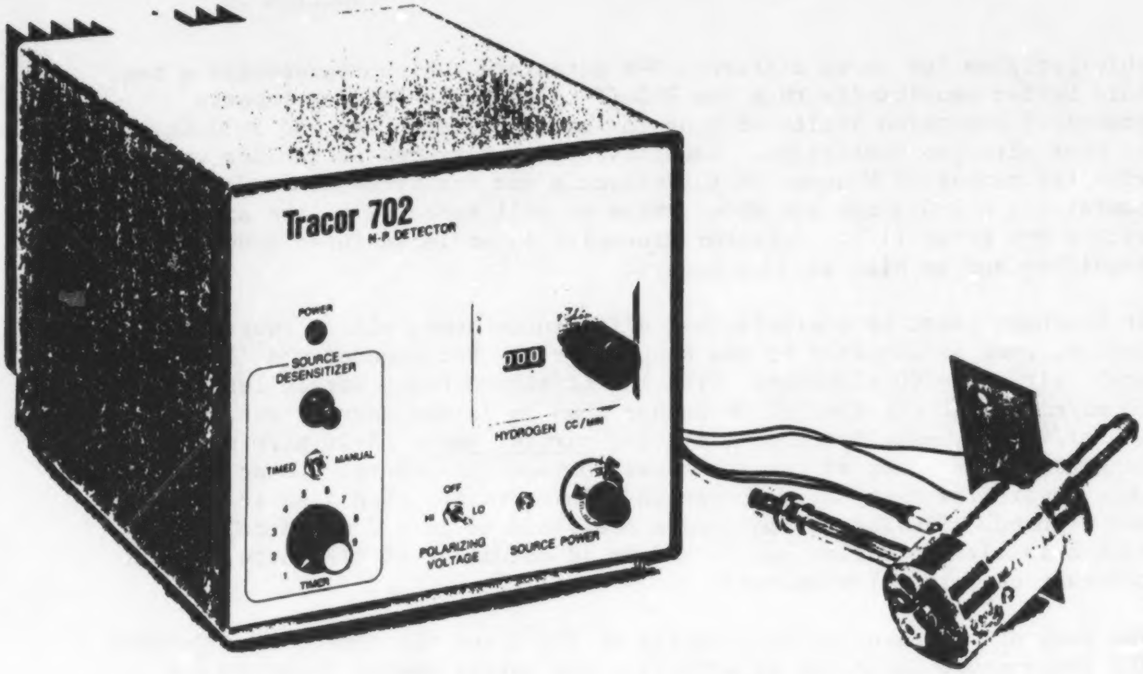

Tracor 702
N-P DETECTOR
POWER
SOURCE DESENSITIZER
TIMED
MANUAL
TIMER
OFF
HI
LO
POLARIZING VOLTAGE
SOURCE POWER
HYDROGEN CC/MIN

Figure 5-J. N-P detector mounting detail for Tracor Model 560

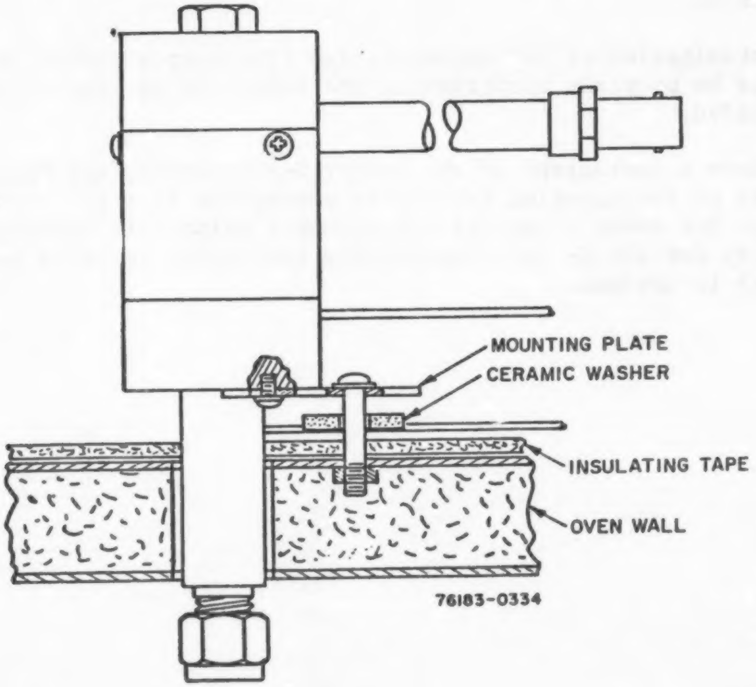
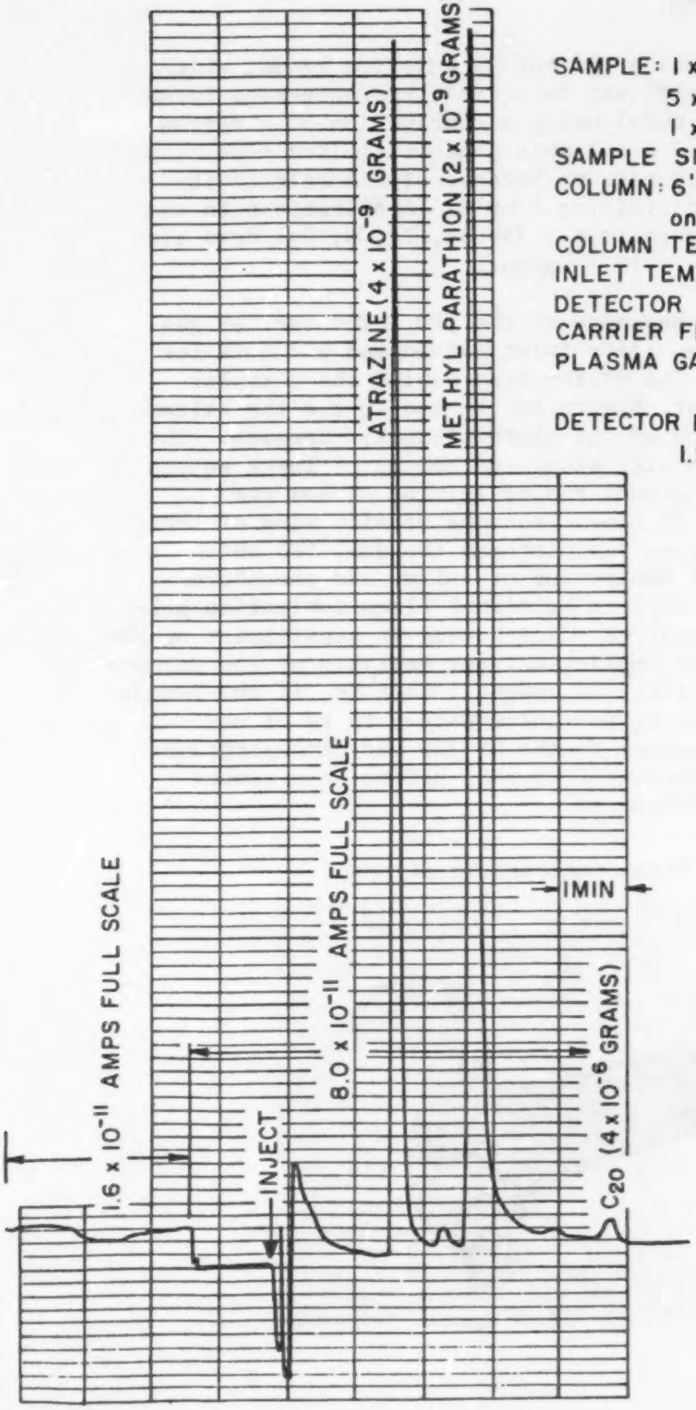

MOUNTING PLATE
CERAMIC WASHER
INSULATING TAPE
OVEN WALL
76183-0334

Figure 5-K. Typical chromatogram of pesticides with the N-P detector.



SAMPLE: 1 x 10^{-9} g/μl Atrazine
5 x 10^{-10} g/μl Methyl Parathion
1 x 10^{-6} g/μl Eicosane in Hexane
SAMPLE SIZE: 4.0 μl
COLUMN: 6' x 1/4" Glass - 3% OV101
on Chromosorb W-HP (80-90mesh)
COLUMN TEMPERATURE: 200°C
INLET TEMPERATURE: 215°C
DETECTOR TEMPERATURE: 250°C
CARRIER FLOW: 25cc/min Helium
PLASMA GAS FLOWS: 2.5cc/min Hydrogen
120cc/min Air
DETECTOR BACKGROUND CURRENT:
1.5 x 10^{-11} amperes

5F FLAME PHOTOMETRIC DETECTOR (FPD)

This detector operates by monitoring HPO and S_2 emission bands, which result from burning the column effluent in a cool, hydrogen-rich flame, at 526 nm (P-mode) or 394 nm (S-mode) using a combination of a narrow band-pass interference filter and a suitable photomultiplier tube. Samples require relatively little cleanup because of the selectivity of the detector for pesticides containing P or S. Applications to the detection of certain other elements (e.g., Ti, As, Zr, B, Cr) have also been made with limits of ca 10^{-7} - 10^{-11} grams.

Figure 5-L shows the external appearance of the FPD. The carrier gas exit line is seen on top and the O_2/air inlet connection and hole for the heater on the bottom side. The mirror lies behind the circular bulkhead seen covering the burner chamber on the front, and the filter lies behind the screw seen on top of the photomultiplier housing. The hydrogen gas inlet is on the opposite side, and column effluent enters underneath the burner chamber. Signal and polarizing cables are attached at the back end of the PM tube. A cross section view of the FPD is shown as Figure 5-M. Figure 5-N pictures the dual FPD which in principle allows simultaneous monitoring of sulfur and phosphorus output from a single injection, as well as normal flame ionization output if it is of interest. In practice, differences in sensitivity of the P and S modes make dual operation impractical for analysis of low amounts of residues where maximum sensitivity is sought. That is, if the P-mode is optimized, the S-mode will not be sensitive enough to be of use. Figure 5-A shows a Tracor FPD mounted to the MT-220 gas chromatograph. At least three other companies produce FPDs that differ in a number of construction aspects and performance.

Figure 5-L. Tracor flame photometric detector

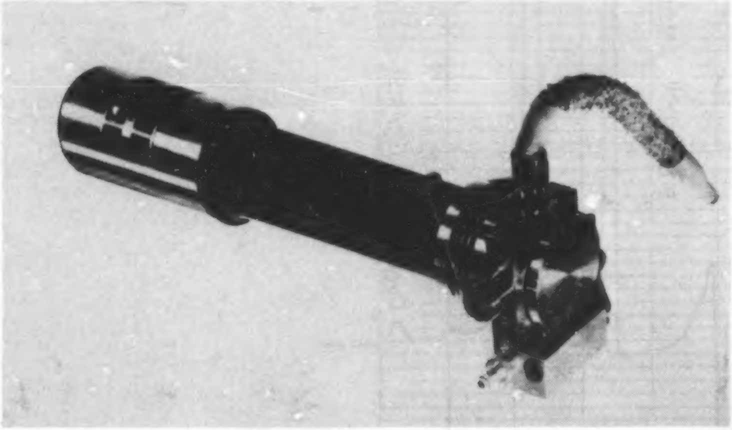


Figure 5-M. Cross section of a flame photometric detector

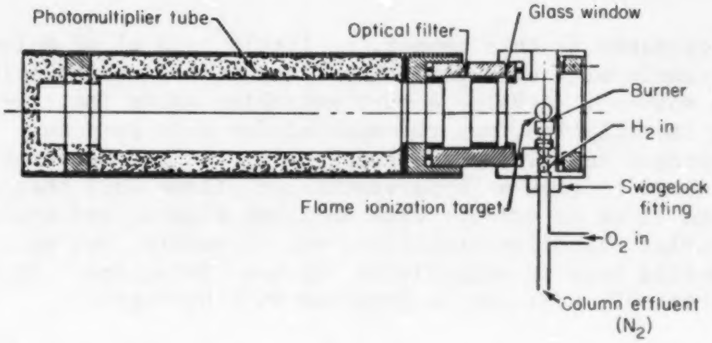
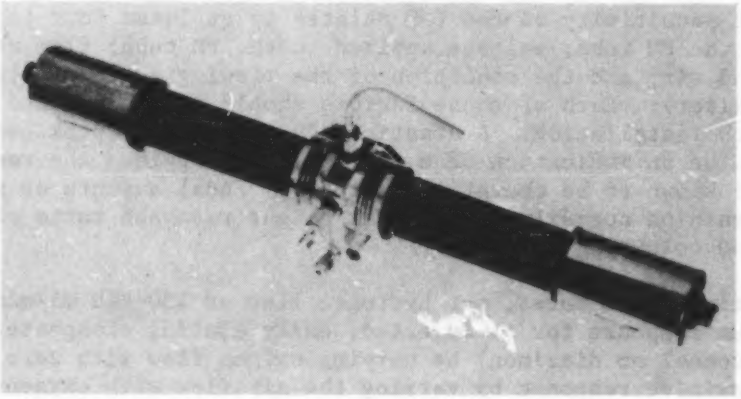

Photomultiplier tube
Optical filter
Glass window
Burner
H_2 in
Swagelock fitting
Flame ionization target
O_2 in
Column effluent (N_2)

Figure 5-N. Dual flame photometric detector



In the original operating configuration of the FPD, oxygen (± air) is mixed with the nitrogen carrier gas and pesticide at the entrance to the detector; hydrogen is brought directly to the burner. Components are burned in a hollow tip that shields the flame from view by the photomultiplier (PM) tube. Emission occurs above the flame tip, and the light is transmitted to the PM tube through a filter that transmits a specific wavelength of the element to be monitored. A potential is applied to the PM tube, and its output is amplified by the electrometer and read-out on a recorder.

When the FPD is operated in this manner, as little as 3 µl of solvent in the injected sample will extinguish the flame unless modification is made to vent the solvent. A Valco 4-part switching valve (No. CV-8HT), silylated before installation, was recommended for this purpose. Reversal of the hydrogen and air/O_2 gas supply lines at the detector inlets has been shown to give a "hyperventilated" flame (30) that allows injection of up to 25 µl of solvent with no flame blowout and similar or better sensitivity, baseline stability, and linearity, but an approximately 20-fold loss in selectivity for most detectors. In this "reverse configuration", effluent is premixed with hydrogen.

The minimum detectable quantities of the elements S and P reported in different sources are about 40 pg-1 ng and 10-100 pg, respectively. In routine operation, 2.5 ng of ethyl parathion should yield a peak height equal to 1/2 fsd, although sensitivity can usually be improved well beyond this in most analyses by careful adjustment of operating parameters. In a comparative study (15), the limit of detection for chlorpyriphos was 115 pg and 174 pg in the P-mode for normal (flame-out) and reverse gas flow configurations, respectively, and 167 and 87 ng in the S-mode with the two flow configurations.

The degree of sensitivity of the FPD relates to at least four factors; condition of the PM tube; voltage applied to the PM tube; flow rates of H_2, O_2, and air; and the condition of the viewing system (optical window and filter); Each of these factors should be checked and optimized for each installation. A drastic reduction in the peak height of malathion can be an indication of a poor column, provided the rest of the system is known to be operating properly. Equal amounts of malathion and ethyl parathion normally give a peak height response ratio of about 0.70 on a good column.

To obtain optimum flow rates, set hydrogen flow at 150-200 ml/minute, obtain maximum response for an injected, early eluting phosphate pesticide (e.g., ronnel or diazinon) by varying oxygen flow with zero air flow, then maximize response by varying the air flow with oxygen set at its optimum value. Some detectors may show best response with no air flow. Maximum response is indicated by a large signal to noise ratio; an increase in flow rates may increase peak height while also causing an increase in baseline noise. Generally, a high flame temperature resulting from too much oxidizer and too little fuel will give the poorest sensitivity. Typical operating parameters for the FPD in the P-mode are in Table 5-2. These values are for the Tracor FPD, based on recommendations in the manufacturer's detector manual and experience in EPA laboratories. Table 5-3 lists FPD parameters used in analytical laboratories participating in the Canadian Check Sample Program on Pesticide Residue Analysis (6). These values were chosen to produce in the S-mode an exponential response factor of 1.9, and to give maximum response in the P-mode. The Tracor company (Personal Communication, 1980) recommends the introduction of 60-100 ml/minute of hydrogen through the air/O_2 inlet and 80-150 ml air into the port marked for oxygen. The use of oxygen is not considered necessary by Tracor with this reversed plumbing arrangement.

The temperature gradient between the column and detector is kept as low as possible, and the detector is always heated before the column when starting up a cold system. The detector base is maintained at about 210°C. The nitrogen to oxygen ratio should be ca 4:1, the carrier and purge flow rates should be equal, and the total flow of air, oxygen, and carrier should not exceed 200 ml/minute. A lower total (100-150 ml/minute) usually produces the most favorable signal to noise ratio.

Although the FPD is not as sensitive to gas leaks as the electron capture detector, the flow system of the chromatograph should be tight. Leaks in the hydrogen, oxygen, or air supply can be hazardous from an explosion standpoint. A common cause of sensitivity loss in the FPD is air leaking into the burner chamber causing a change in flame characteristics. This is remedied by replacing the gaskets (probably discolored and brittle) with new, bright, and flexible ones. Stability of the FPD is promoted by having a very stable flame. This is best accomplished by using high quality flow controllers for the detector gases.

Optimum response voltage for the PM tube is determined using a variable power supply that allows the voltage to be increased with little increase in electronic noise. Raising the voltage from the electrometer will increase electronic noise inordinately. With optimum flow rates, the power supply is set at 750 V, and a sample of diazinon is injected to give 30-60% fsd. The injection is repeated at 850 V and at voltage increments between and around these values until the point of maximum signal to noise ratio is determined. It may be necessary to attenuate to keep on scale during this determination, so the linearity of the electrometer must be known. Different PM tubes require different voltages for best performance, a value of about 850 V being typical. A suspect PM tube may be checked with one of known sensitivity to give indication of its condition. Satisfactory operation of the FPD over its full dynamic range requires both a highly stabilized 750 V power supply plus an electrometer with a bucking capability at least 1×10^{-6} amps. PM tubes are heat sensitive and should be well insulated from sources of heat in order to keep sensitivity from being lost. The tube can be kept cool by blowing air over it or circulating cooling water through a copper tube wrapped around it. In a modified FPD design that protects the PM tube from heat radiation, emitted light is carried to the phototube by a light-pipe.

Table 5-2

OPERATING PARAMETERS FOR THE P-MODE OF THE TRACOR FPD

Temperatures (^{o}C)		Flow Rates (ml/min.)	
Column	200	Purge*	70-80
Injection Block	210-225	Carrier	70-80
Detector**	165-250	Hydrogen	50-200
Transfer Line	235	Oxygen	10-30***
Switching Valve*	235-240	Air	0-100

* With Valco switching valve

** High temperature model is never heated above 250°C, low temperature model never above 170°C

*** To ignite the flame, an oxygen flow of 50 ml/minute or more may be required, depending on the detector

Table 5-3

GAS FLOW PARAMETERS SUGGESTED FOR OPTIMUM RESPONSE WITH THE MELPAR (TRACOR) FLAME PHOTOMETRIC DETECTOR (6)

	Old configuration		New Reverse Configuration	
	P-mode	S-mode	P-mode	S-mode
H_2 (ml/min)	200	70	200	50
Air(")	80	30	30	50
O_2 (")	10	10	15	10
O_2/H_2 ratio	0.13	0.22	0.11	0.4

Interference filters may be changed at any time, i.e., it is not necessary to shut down the instrument to do this. The power to the PM tube must be turned off when removing it from the flame base. Excess light will damage or destroy the sensing element when the tube is connected to the power supply. Light leaking into the PM tube during operation of the chromatograph will increase the noise level and decrease sensitivity. One remedy is to tighten all connections having gaskets between the burner and the mounting of the PM tube. A second remedy is to construct an opaque fiber or plastic tube to slip over the connection of the PM tube and the metallic link with the cooling fins, or a light-tight box to completely house the burner and PM tube. Ignition of the flame may be detected by observation of recorder pen deflection up or down scale, hearing the "pop" of the hydrogen gas, or deposition of moisture on a cold, flat metal surface held near the exhaust tube. After several months of operation, the quartz window in the FPD burner becomes pitted and "fogged" or opaque. This loss of transparency can cause a decrease in sensitivity that can usually be restored by polishing the window with carborundum or jeweler's rouge and a polishing mat commonly used by infrared spectroscopists for salt windows. If the pitting or fog is too deep and cannot be polished out, the window should be discarded and replaced.

The FPD response to P is linear over a concentration range of about 3-5 decades, e.g., 0.4-400 ng for parathion and 0.14-400 ng for chlorpyriphos (15) in the P-mode. Nonlinear response of the FPD (526 nm filter) to oxygen analogs of OP pesticides is often noted and is thought to be caused by degradation of these P = O compounds. GC columns should be optimized for separation of these compounds without breakdown, and metal transfer lines between the column and detector should be as short as possible and preferably made from Teflon or glass-lined metal tubing. The S-mode is inherently less sensitive than the P-mode, and response for compounds containing a single S atom is nonlinear starting in the 1-10 ng range. The response increases very roughly as the square of the concentration of sulfur, so standard curves are plotted on semi-log paper for S-mode quantitation. Quantitative evaluation of chromatographic data from the nonlinear S-mode FPD has been theoretically and experimentally studied (31). A linearizing amplifier option is available for commercial detectors that electronically transmits the square root of the detector response to the recorder so that plots of peak height or area vs concentration are linear within ± 5%. This linear response facilitates easy interpretation and allows electronic integration and data acquisition not possible without the square root function. The potential errors involved in the use of these commercial linearizers, if response is not actually proportional to the square of S concentration, have been evaluated and recommendations made to minimize the error (32).

The unique square-law sulfur response of the FPD can be used to help distinguish sulfur pesticide peaks from interfering peaks due to large concentrations of nonsulfur compounds, such as hydrocarbons, that can also be detected. Because the peak height of sulfur compounds varies as the square of the sulfur atom flow into the FPD, peaks due to sulfur compounds tend to be narrower than those of nonsulfur compounds with comparable retention times. Therefore, visual inspection can often identify these unusually narrow peaks. A more definite identification will be achieved if the volume of sample injected is increased. Peak heights of sulfur compounds will increase as the square of the injected volume while peak heights of nonsulfur compounds will increase linearly. As a result, the sulfur compounds effectively rise up out of the background. If the hydrocarbon peaks are not well resolved from the sulfur compounds, hydrocarbon quenching of sulfur light emission may diminish the advantage of this square-law response.

In order to operate in the dual mode, it is necessary to optimize combustion gas flows for the S-mode and to have sufficient sulfur to detect in this mode. This combustion mixture is not necessarily the optimum for best phosphorus response. Optimum conditions will vary from detector to detector. If enough residue is present to detect in the S-mode, attenuation must be used to keep the P response on scale with the S response.

The proper attenuation for a given sample will depend upon the sensitivity achieved, but, in general, it is best to operate near the maximum and to dilute the sample as necessary. Selectivities for P and S are about 10,000-25,000 or more:1 compared to nitrogen, carbon, hydrogen, and oxygen. Large amounts of sulfur impurities give a response in the P-mode (P:S response ratio 4-25:1 at 526 nm) whereas phosphorus impurities cause negligible response in the S-mode (S:P response ratio 100-1,000:1 at 394 nm). As the degree of sulfur oxidation in the molecule increases, there is usually a decrease in sulfur response. Factors affecting selectivity of the FPD have been studied (33).

Maximum utility of the FPD is afforded by the dual photomultiplier arrangement (Figure 5-N) whereby P and S are simultaneously monitored on a dual-pen recorder. This arrangement informs the analyst whether a compound contains only P or S, or both, and the P/S ratio (the P-response divided by the square root of the S-response) is important information for confirmation of residue identity. The response ratio ($x10^3$) ranges from 5.0-5.8 for PS compounds, 2.5-3.4 for PS_2 compounds, and 1.6-2.4 for PS_3 compounds (34). As mentioned earlier, dual operation will not be practical for analysis of low amounts of residue barely detectable by the P-mode of an FPD optimized for this mode because of the much lower sensitivity of the S-mode under these conditions.

Errors have been noted (35) in quantitations with the FPD in the P-mode when automatic integration is applied. The detector response passed through a minimum after the solvent peak and then gradually rose to the baseline without passing through a maximum to stop the integration before the first pesticide peak. This was overcome by adding a low boiling organophosphate (e.g., tributyl phosphate) that eluted after the solvent but before the pesticide peak (malathion was studied). The FPD has been coupled with a capillary GC column for analysis of OP pesticides (36).

The FPD has proven to be a versatile, sensitive, selective, and reliable means of analyzing not only pesticides and metabolites containing P and S atoms, but also for compounds such as carbamate insecticides in the form of derivatives containing these elements. The FPD has advantages over the normal flame thermionic detector for routine analysis in terms of ease of operation, better stability, less maintenance time, independence of response to gas flow rates, and need for less frequent injection of standards. Sensitivity of the FPD is about one order of magnitude less for P compounds than with a fully optimized flame or flameless thermionic detector. Applications and limitations of the FPD in atmospheric analysis have been reviewed (5).

Varian has developed a new FPD with dual flame design that is reportedly (37) superior, but it has not yet been carefully evaluated for routine residue analysis. Two hydrogen-air flames are used to separate the regions of sample decomposition and emission, so that the upper emission flame is more efficient, and sensitivity is improved compared to the single-flame FPD. The major claimed advantage of this construction is the reduction of the effect of hydrocarbon background quenching of the light emission from S- or P-compounds, because much of the C-H emission takes place in the lower oxygen-rich flame, while only the upper hydrogen-rich flame is viewed by the photomultiplier. Reported selectivities are 10^5 grams C/gram P and 10^3 grams - 10^6 grams C/gram S, and response is less affected by the compound structure because of more complete breakdown into S_2 and HPO species. Up to 200 µl of solvent can be injected without extinguishing the flame, and a pushbutton linearizer for the exact quadratic response of the S-mode is included.

5G ELECTROLYTIC CONDUCTIVITY DETECTOR

This detector operates by mixing of the column effluent with oxygen or hydrogen reactant gas, followed by oxidation or reduction in a furnace containing certain catalysts. In the original Coulson detector, ionizable species emanating from the combustion zone are contacted with deionized water, and the carrier gas is separated from the liquid in a separator. The conductivity of the water is changed due to the presence of the ionized species, and the change is measured and displayed on a recorder in the form of usual GC peaks. Table 5-4 shows the various modes of operation of the Coulson conductivity detector as described by Cochrane. The conditions and selectivity would be similar for the Hall detector. Selectivity and sensitivity in these modes are governed by the furnace temperature, nature and flow rates of the reactant and carrier gases, flow rate of water through the cell, and proper choice of catalysts and scrubbers. Each analyst must optimize his conditions for the compound in which he is interested. As a result, the minimum sensitivity values reported by different workers for compound classes have varied quite widely. A general review of the electrolytic conductivity detector has been published (38).

Gas chromatography with the Hall electrolytic conductivity detector (HECD) is described in Sections 4,C,(1)-(5) of the EPA PAM. Included are discussions

TABLE 5-4

CONDUCTIVITY DETECTION MODES OF OPERATION - COULSON CONDUCTIVITY DETECTOR

Mode	Conditions	Selectivity	Sensitivity
N	H_2 reduction gas - 850°C - Ni wire catalyst - NH_3 formed and detected as NH_4+ - He or Ar carrier gas	$Sr(OH)_2$ scrubber removes acidic products - responds only to N compounds	100 pg N; 100-200 ng subst. ureas, 7-15 ng triazines, 50-80 ng carbamates, 35-50 ng thiol-carbamates; 150 ng parathion, 25 ng diazinon.
Cl	empty tube reduction using H_2 gas - HCl produced - 600 or 850°C - N_2 carrier gas	responds to Cl and N-contg. OP insect. such as diazinon	500 pg Cl; 6-40 ng Cl insecticides.
S	S-compounds oxidized to SO_2 and SO_3 in O_2 stream in empty tube - Cl converted to HCl - N_2 carrier gas - Pt gauze catalyst may be used - Furnace temperature 850°C	responds to S, Cl, and N - Ag wire scrubber increases selectivity with respect to Cl, but at expense of sensitivity and reproducibility - CaO scrubber for SO_2 has also been used	1 ng S; 30-2200 ng N compounds gave 1/2 fsd, depending on O_2 flow.
Pyrolytic	empty tube pyrolysis 400-600° or 850°C	responds to N, Cl, S	500 pg S and/or Cl, 1 ng N; linear range 2-1000 ng S; 6-100 ng S and Cl pesticides.

From: Cochrane, W. P., presented at May, 1973 Symposium on Pollutant Analysis, Athens, Ga.: See also J. Chromatogr., 75, 207 (1973); Int. J. Environ. Anal. Chem., 3, 199 (1974); and (28).

of the GC instrument, choice of columns, methods of quantitation and interpretation, GC data and chromatograms, and a complete description of the principles and details of operation of the various modes of the Tracor Model 700 detector. The FDA PAM also contains material on the Hall detector, in Section 315.

The current commercial version of the Hall detector is shown in Figures 5-O and 5-P. Figure 5-O displays the basic components of the 700A detector and conductivity cell, while Figure 5-P shows the appearance of the commercial detector. The Model 700A is available only with the Tracor Model 560 gas chromatograph. The Model 700 is similar to the 700A and can be connected to other gas chromatographs. The Model 700A features precise electrolyte flow regulation, a microreactor furnace, extremely low dead volume, improved scrubbers, and automatic solvent venting. A new differential conductivity cell design combined with a bipolar pulse cell excitation system provides increased sensitivity compared to older models.

A comparison of the Hall and Coulson detectors has been carried out. An approximate 7-fold increased sensitivity was found for the Hall detector relative to the Coulson detector for nitrogen-containing pesticides. Values obtained on a 4% OV-101/6% OV-210 column at 205°C were as follows (40):

	ng for 1/2 fsd	
	Coulson	Hall
Atrazine	7	1.1
Bladex	15	1.1
Chloropropham	75	6.0
Diazinon	25	4.5
Ramrod	50	6.5
Parathion	150	20

The Tracor company reports sensitivities of 40 ng of atrazine (N-mode) and 40 ng atrazine and 20 ng aldrin (Cl-mode) for 30-60% fsd peaks with <1% fsd background level for the 700/700A detectors under the following typical operating conditions: 1.8 m x 6.4 mm 3% OV-1 column, 200°C, helium carrier 50 ml/minute, hydrogen reaction gas 50 ml/minute, 50% n-propanol in deionized water electrolyte flowing at 0.8 ml/minute, 850°C furnace temperature (41). The improved sensitivity of the Hall

Figure 5-O. Cross section of HECD microreactor and conductivity cell (39)

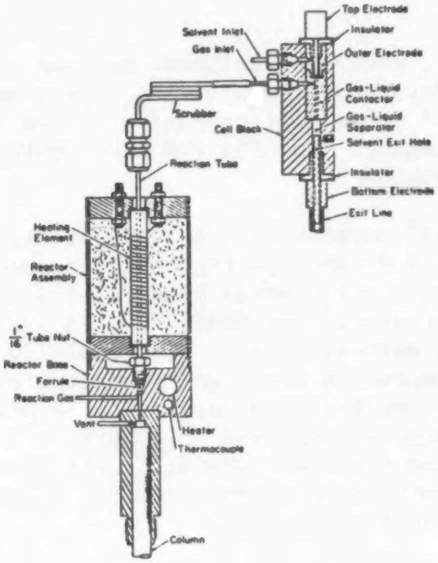
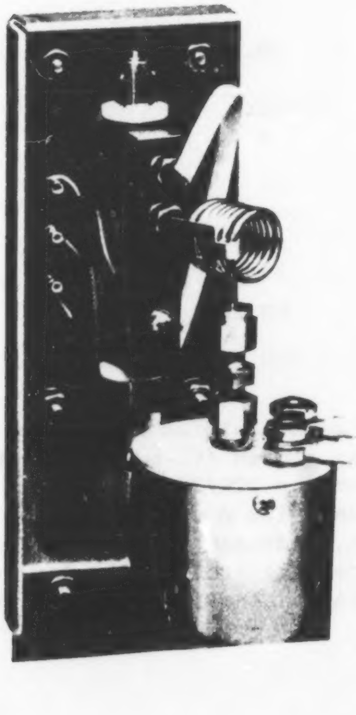
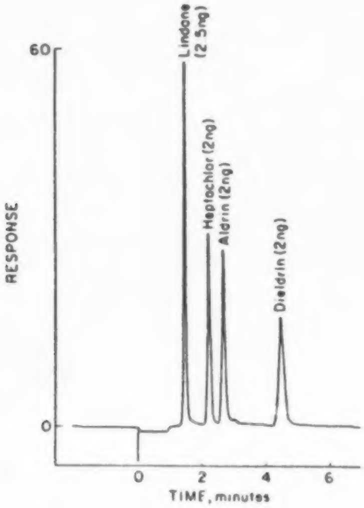

Top Electrode
Insulator
Solvent Inlet
Gas Inlet
Outer Electrode
Gas-Liquid Contactor
Scrubber
Gas-Liquid Separator
Cell Block
Solvent Exit Hole
Reaction Tube
Insulator
Bottom Electrode
Exit Line
Heating Element
Reactor Assembly
$\frac{1}{16}$" Tube Nut
Reactor Base
Ferrule
Reaction Gas
Vent
Heater
Thermocouple
Column

Figure 5-P. Tracor Model 700A Hall electrolytic conductivity detector



detector is seen by comparison of these values with Table 5-4. As seen in Figure 5-R, experimental detection levels are often well below these reported amounts.

Figure 5-R. Chromatogram obtained with the Hall electrolytic conductivity detector in the Cl-mode (2)



Selectivity values are $Cl/C > 10^6$, $N/C > 10^5$, and $S/C \geq 10^5$, and linearity for Cl is 10^5-10^6, for N 10^4, and for S > 10^4. Figure 5-S demonstrates the sensitivity and selectivity of the N-mode, while Figure 5-T shows the chromatogram of spiked soil extracts (20 grams soil/40 ml methanol) injected without cleanup. Figure 5-U shows the sensitivity and selectivity for sulfur detection with the catalytic/oxidative mode; the pyrolytic/oxidative mode can also be used for S-detection with about one order of magnitude superior selectivity. Figure 5-V shows the S-mode analysis of lettuce extract (acetone) without cleanup.

Figure 5-S.
HECD 700A selectivity
in the N-mode (39)

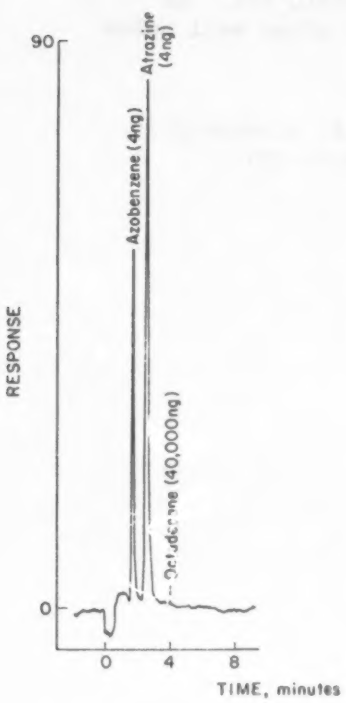


Figure 5-T.
Soil extract (A and B)
with atrazine added, and
(C) control (39)

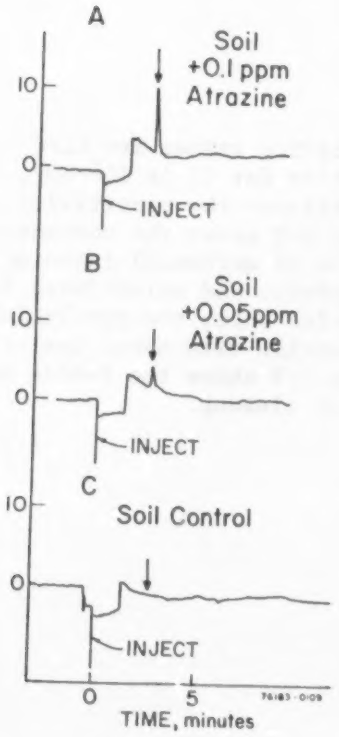


Figure 5-U. HECD response to hydrocarbon and sulfur compounds (39).

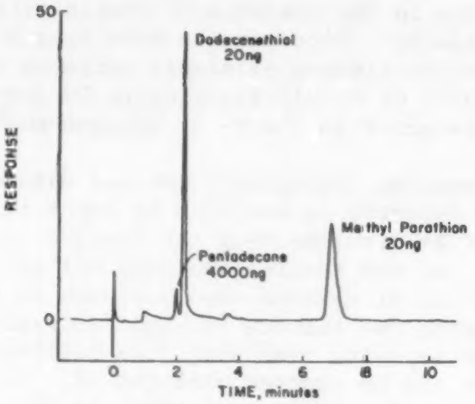
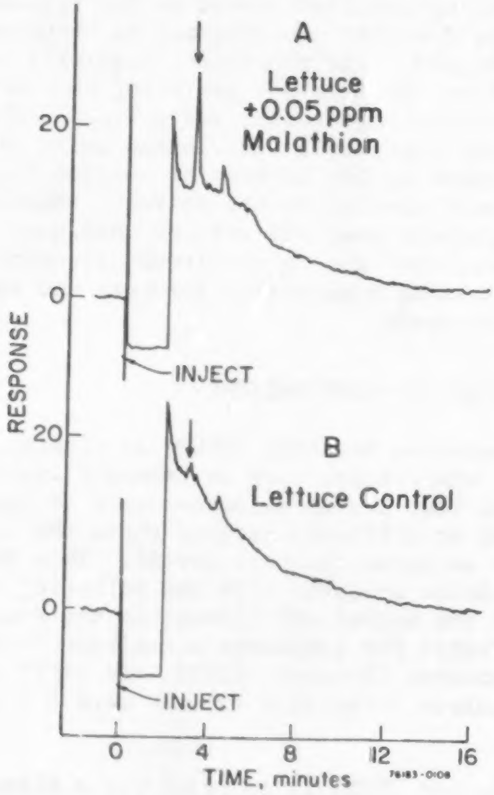


Figure 5-V. Lettuce extract with malathion added (A) and control (B) (39)



EPA experience has been that detection sensitivity of the Hall detector is lost in some laboratories in the analysis of sample extracts compared to results with standards. Good results have been obtained when gel permeation chromatographic cleanup of sample extracts is combined with the Hall detector. QF-1 or OV-210 fluorinated GC liquid phases may not be used with the detector in the N- or halogen-modes (42).

The following are some operating characteristics and maintenance instructions for the Hall detector as outlined by Bayer (43): Cleaning requirements are minimized by disconnecting the furnace to cell transfer line, leaving the furnace on, and turning the pump off at the end of the day's analyses. Build-up of carbonaceous residues in the quartz tube is alleviated by running the furnace at high temperature in the oxidative mode. Siliceous deposits resulting from silicon column bleed or silyl derivatives can be removed with 10% HF. Alternatively, the quartz tube can be replaced. Small variations in the conductivity solvent flow rate will change the detector response, so the flow rate should be set to a constant value each day. The recommended 1-5 cc/minute hydrogen flow rate through the standard 2 mm id quartz tube is very difficult to achieve in the reductive mode with the supplied needle valve, but variations have only minor effects on detector sensitivity. The maintenance and cleaning required depend on the type of samples analyzed. Weekly or more frequent cleaning may be required if dirty samples are commonly analyzed. The procedure, requiring less than one day, involves disassembling the unit and replacing the quartz tube, Teflon transfer line, ion-exchange resin, and solvent. The needle valve, its filter, and the conductivity cell are cleaned in an ultrasonic bath. Baseline noise can be caused by air bubbles or residue trapped in the conductivity cell and ionic species in the solvent. Bubbles are removed by rapidly turning the solvent pump off and on. Residues are removed by disassembling and cleaning the cell in an ultrasonic bath, and ionic species are minimized by using high purity solvents and water and routinely changing the ion-exchange resin.

5H OTHER DETECTORS AND DETECTOR COMBINATIONS

The sulfur-phosphorus emission detector (SPED) is similar to the FPD except that fiber optic bundles are used to transmit light from the flame to the PM tube and that the chemiluminescence of the HPO and S_2 species are monitored at different heights above the flame (the viewing port for P is 6 mm above the port for S). This detector has been evaluated for pesticide analysis with the following results (28): response was similar to the normal FPD (linear in the P-mode, squared in the S-mode, and quadratic for compounds containing both P and S); linearity for three standards (Ro-neet, DEPPT, and DEPP) in the P-mode was 10^2-10^3, and the minimum detectable amounts were 5×10^{-11} to 2.3×10^{-13} g/second.

The photoionization detector (PID) is in principle a flame ionization detector in which the ions are created by UV photons instead of a flame.

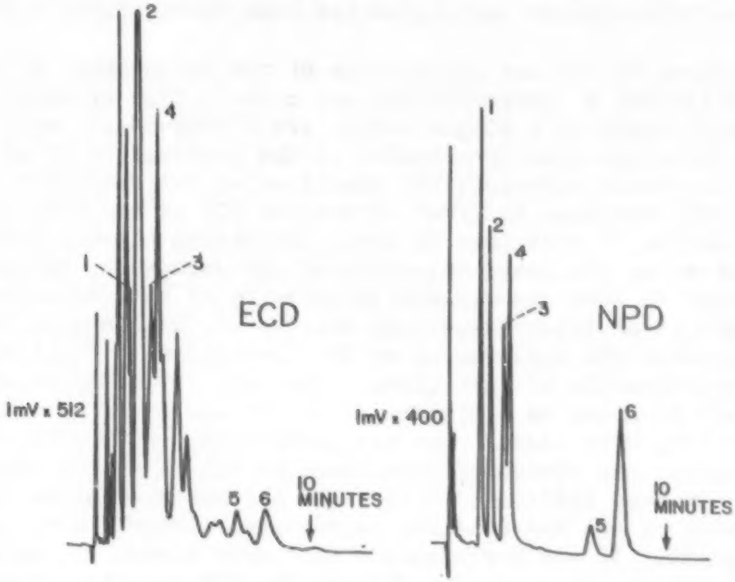
Sensitivity is 10 to 50 times greater and the linear dynamic range is 10-100 fold greater than the FID. The PID is sensitive to inorganic compounds such as NH_3, PH_3, and AsH_3 to which the FID does not respond. The detector (44) has a sealed UV source focused directly into the ionization chamber. The UV energy photoionizes the various compounds eluting from the GC column but not the helium carrier gas. Organic compounds with ionization potentials greater than the 10.2 ev energy of the source, such as C_1-C_4 hydrocarbons including many common pesticide solvents (methanol, methylene chloride, carbon tetrachloride, acetonitrile), do not respond. The increased sensitivity is apparently due to the increased ionization efficiency of photons compared to a flame and operation of the PID in an oxygen-free environment, thereby eliminating free-radical quenching. The linear dynamic range has been reported as $10^7 - 10^8$, and the minimum detection level was <2 pg for benzene. The PID response to carbon is proportional to the carbon number as is the FID. Because it responds to the sample concentration, maximum sensitivity is obtained at low flow rates (1-10 to 10-100 ml/minute). The maximum operating temperature is 315°C. The nondestructive detection of ng levels of organophosphorus pesticides has been demonstrated (45).

Principles of the use and details of the arrangement of multiple detectors for efficient GC determination and confirmation of pesticides of different chemical types in a single sample are discussed in Section 320 of the FDA PAM, and a specific description of the combination of electron capture and thermionic detectors for simultaneous determination of OCl and OP pesticide residues is given in Section 321 of the same manual. When a combination of detectors is used, the chosen cleanup procedure must be suitable for the least selective of the detectors, enough residue must be injected to meet the minimum sensitivity of both detectors, and the nature and amount of injected solvent must be compatible with both. Figure F-W illustrates the application of dual detectors to facilitate identification and quantitation of pesticides. The left chromatogram shows the electron capture response to a mixture of six N- and/or P-containing pesticides to which PCBs were added. The six pesticides, the positions of which are indicated, are obviously overlapped by the PCBs to a degree that makes analysis very difficult. The right chromatogram shows the selective response of the N-P detector to the same mixture, with no detection of the PCB peaks. If the N-P detector were used alone, the presence of the PCBs would not be ascertained. Without the N-P detector, the pesticides could not be properly determined. Therefore, both detectors are useful for this sample (2).

A GC system with one column, a three-way effluent splitter, and five different detectors [electrolytic conductivity (N-mode), FPD (FID, S-, and P-modes), and electron capture] operating simultaneously was described (46). A computer program for evaluating data from this system was later published. The program calculates retention times relative to two internal standards as references and peak areas corrected for baseline drift (47).

The mass spectrometer (Section 10L) can be used either as a universal detector or the ultimate specific detector for gas chromatography. For the latter purpose, specific ion monitoring at a single mass number or simultaneous monitoring of several selected characteristic fragment ions is carried out (48). Linearity of response generally extends over several orders of magnitude. Picogram sensitivity has been achieved, even at high resolution (49).

Figure 5-W. Analysis of a pesticide mixture in isooctane solution to which a small amount of polychlorinated biphenyl mixture was added, by gas chromatography with two different detectors. Column: 1.8 m x 2 mm id glass, packed with 3% OV-101 on Gas Chrom Q, 80-100 mesh. Column temperature: 190°C. Sample volume: 1 μl containing each of the six pesticides in the amount of 5 ng. Peaks: 1-di-syston, 2-methyl parathion, 3-malathion, 4-parathion, 5-methyl trithion, 6-ethion (2).



5I ELECTROMETER AND RECORDER

The electrometer is primarily a device for amplifying the electrical signal from the detector prior to its introduction to the recorder. Units may be single channel, designed to operate with one detector and recorder, or dual channel. Customary controls on the electrometer include input and output attenuators, output polarity, and controls for the recorder zero and bucking current. Servicing of electrometers is generally a function of a trained electronic technician or representative of the company manufacturing the chromatograph.

To check electrometers on the Tracor MT-220 chromatograph, set attenuators to the off position and zero the recorder. Set the output attenuator at x1 and record the baseline. A steady baseline with less than 1% noise should be obtained.

Recorders may be of single or dual channel design, the latter being capable of receiving two separate voltage signals supplied from the electrometer to two pens that trace separate chromatograms on opposite sides of the same chart paper. Electronic controls on a recorder usually consist of a pen zero and a signal gain adjustment. Most chromatographs require a recorder with a full scale sensitivity of 1 mv and a full scale response of one second or less.

Proper adjustment of the recorder gain control is extremely important. Some analysts, upon observing excessive baseline noise, erroneously conclude that this should be eliminated by lowering the gain. When the gain is set too low, however, the resulting chromatograms appear "terraced" with a stepping-stone effect in the baselines. In extreme cases, peaks have jagged and flat rather than pointed tops. When this is evident, correction can usually be achieved by advancing the gain control to a point just short of pen chatter.

5J SAMPLE INJECTION AND THE INJECTION PORT

a. On-Column and Off-Column Injection

Some gas chromatographs have injection ports designed to accommodate either on-column or off-column injection. The former entails insertion of the syringe needle directly into the glass wool inlet plug of the column. For off-column injection, some type of glass or metal insert is installed in the injection port, and injection is made into this insert where the sample is flash vaporized and swept into the column by carrier gas. In practical operation in a pesticide laboratory that is injecting a heavy volume of biological extracts, off-column injection through a glass insert is preferable. A significant amount of extraneous material that would otherwise be injected directly into the column is trapped by the insert. If your chromatograph does not provide the option of off-column injection, it is mandatory to frequently change the glass wool inlet plug. The frequency of change is determined by daily monitoring of the extent of p,p'-DDT conversion (see 4F of Section 4). The plug is changed when the combined areas of the breakdown peaks (DDE and/or DDD) exceeds 3 or 4% of the sum of the areas of the p,p'-DDT and the breakdown peaks.

An earlier discussion of some problems associated with injection of unclean samples and maintenance of the injection sleeve was presented in Subsection 4F. Glass injection sleeves are cleaned in chromic acid cleaning solution, rinsed with water and acetone, and dried. A final silanization treatment of the clean injection sleeve with Supelco's Sylon-CT has also provided a dramatic solution to p,p'-DDT and endrin breakdown problems. The label instructions were followed.

There are known instances where changing the insert, glass wool plug, and even the first 1/2 inch of packing have not diminished conversion of DDT resulting from massive injections of uncleaned samples without proper on-going maintenance. Final correction required disassembly of the entire injection port and wire brush cleaning of all metal parts to remove encrusted filth. Following this, each part was further cleaned in an ultrasonic cleaner in alcohol KOH and finally acetone rinsed. Some analysts recommend the use of a small plug of quartz wool in the exit end of the injection insert to act as a further trap for extraneous contaminants.

b. Septa

A large number of different types of septa are available commercially including inexpensive silicone rubber designed for low temperature, routine GC; high temperature silicone rubber; and expensive, layered or sandwich types. Catalogs of the different suppliers should be consulted for the specifications of the available products.

The septum chosen for residue analysis should not produce significant bleed (ghost peaks) that can affect identification of quantitation of residues under the conditions used for gas chromatography. Bleed generally increases as the inlet temperature increases and diminishes with the length of time the septum has been installed. A leaking septum may cause a number of problems, including baseline drift, loss of sensitivity or erratic sensitivity, increase in peak retention times because of loss of carrier gas, or column deterioration due to entering air.

Septum leaks can be caused by loss of elasticity as a function of temperature and time of use, injection with a large diameter or damaged (bent or burred) syringe needle, or incorrect tension of the septum nut. It is preferable to change the septum before a leak develops to prevent the production of incorrect analytical results. Change might be made on at least a daily basis if the instrument receives heavy use.

Replacement at the end of the working day is convenient since this will allow the septum to condition during the night and be ready for use the next morning. A needle guide on the syringe or an injection port with a small diameter hole can prolong the life of the septum by causing the needle to penetrate the septum at the same point, thereby minimizing coring and tearing of the septum. A needle guide also helps to maintain the integrity of the needle itself. Overtightening of the septum nut will tend to extrude the septum and increase the amount of septum bleed. Undertightening can reduce the ability of the septum to seal.

The septa in widest use for residue analyses are the high temperature silicone rubber septa such as blue HT (Applied Science), white HT (Alltech Associates), Thermogreen LB-1 (Supelco); perfluoroelastomer type (Pyrosep, Supelco); and layered septa with Teflon or polyimide faces that are placed against the injection port (LC Company). In one comparative study (50), the blue HT tested best for high temperature use and long life. After baking at 300°C in an unused injection port overnight

(15 hours), this septum gave good service for 50 injections using a 26-gauge needle, without leaking or producing ghost peaks. The Teflon faced septum did not produce ghost peaks initially but did bleed after a few injections had ruptured the face and exposed the silicone rubber. After puncturing, the Teflon septum performed more poorly than the blue HT.

In another comparative study (51), the rubbery Teflon perfluoroelastomer septum gave the least bleed of all septa tested, including silicone rubber and Teflon-faced septa. Reports from another laboratory indicate that polyimide-faced septa are superior to those that are Teflon coated in terms of bleed at high temperature, but that the unlayered high temperature rubber type is still preferable.

The method of septum preconditioning depends upon the temperature to be employed. For use below 250°C, rinsing with acetone, wiping with a Kimwipe tissue, and air drying may be sufficient for HT silicone rubber septa. For higher temperature use, overnight baking at 300°C (50) will probably be required. Each laboratory should evaluate its septa (see below), and precondition and replace them as required for its applications. Septa should never be handled with the fingers, but rather with a tissue or clean forceps.

Although different laboratories have individual methods for changing a septum, the following considerations are appropriate. It is undesirable to expose a GC column to air while it is hot. This can cause oxidation of the stationary phase and column deterioration. Also, quick removal of the septum nut while the carrier gas is flowing (column under pressure) can cause the column packing to shift or be blown from the column. To avoid these problems while changing the septum, reduce the column temperature and shut off the carrier gas flow when the column is cool. When the gas flow has ceased, remove the nut and insert the new septum. Resume the carrier gas flow, allow the column to flush for a few minutes, and reheat the column.

The following procedure allows evaluation of septa for high temperature applications: place a clean metal disc in the injection-port nut and install a short (e.g., 46 cm) nonpolar column such as Dexsil 300 on 80-100 mesh Chromosorb W-HP in the gas chromatograph. Heat the injection port to 300°C (or the temperature of interest), set the attenuator to a sensitive setting, and program the oven at 20°C/minute from 50°C to the maximum temperature of the column. No peaks should be detected. If the instrument has a septum purge, turn this off. Cool the oven to 50°C and replace the disc with the septum to be tested. Wait 10 minutes and then temperature program the column as above. If peaks are produced, a preconditioning step such as baking must be used to eliminate volatiles from the septum. Perform this preconditioning on the septum and reevaluate it.

c. Injection Techniques

(1) Handling the Syringe

When a sample is injected into the chromatograph, it is essential that it be entirely vaporized without loss. Injections are usually made using a 10 µl syringe for the electron capture, thermionic, and FPD detectors or a larger capacity if required for other less sensitive detectors (e.g., microcoulometric). Automatic injection devices are available for use with some chromatographs and detectors. (See Section 50k).

Samples are injected from a microliter syringe by inserting the needle through the septum as far as possible, depressing the plunger with the thumb or finger, then immediately withdrawing the needle (keeping the plunger depressed) as rapidly and smoothly as possible. Some analysts prefer a delay of 1-2 or up to 5-10 seconds before withdrawal of the needle. When initially filling the syringe, air is expelled by repeatedly drawing liquid in and rapidly expelling it with the needle tip still under the liquid surface. The volume of sample to be injected is exactly adjusted by drawing up a couple of µl more than necessary into the barrel. Hold the syringe vertically with the needle pointing up, put the needle through a tissue to absorb expelled liquid, and push the plunger until it reads the desired value. The excess air should now have been expelled.

There should be no delay between filling and injection of the sample. After injection, the syringe is rinsed clean by filling with and expelling 5 portions of ethyl acetate or acetone, and the syringe is pre-rinsed with the next sample to be injected in the same manner. Be sure to follow carefully all manufacturer's suggestions for proper use of each particular syringe.

When a sample is injected in this normal manner from a 10 µl syringe, the needle will retain ca 0.2-0.3 µl of sample. It is usually safe to ignore this volume since standards are injected for comparison, and the errors due to retained volumes will cancel out if equal volumes are used and concentration differences are negligible. Alternatively, the syringe may be filled by drawing the entire sample into the barrel, noting the final volume by reading each end of the column of liquid. After injection, the plunger is pulled back and the small volume of retained solvent now in the syringe barrel is read and applied as a correction. This will correct for nonreproducible injection technique but not, however, for the error encountered if the retained volume has a composition different from the original sample, as would happen if nonuniform distillation had occurred in the needle. Then the remaining liquid would be richer in high boiling sample components.

This can be overcome by using the solvent flush injection technique, the most reliable and reproducible method available. About 2 µl of solvent is first drawn into the syringe followed by a 1-2 µl air pocket and then the required volume of sample. The sample is brought completely into the barrel so its volume can be read. On injection, the flush solvent behind the sample ensures injection of the entire sample without loss due to hang-up. Whatever method the analyst chooses to employ, he must be as consistent as possible in his injections of standards and sample. It is critical that the solvent chosen for injection of the sample completely dissolves the residues of interest, and the same suitable solvent should be drawn first into the syringe for the flush technique. The suitability of the solvent should be verified by obtaining reproducible peaks from repeated injections of a sample dissolved in the solvent [see Subsection 5Jc(3)].

It is good practice to reserve one syringe only for electron capture work. If a series of concentration levels is to be injected, the more highly concentrated solutions should be injected last. If the complete freedom of a presumably clean syringe from pesticide traces is suspect, pure solvent should be injected and any peaks would indicate contamination and need for further cleaning. Dirty syringe plungers and needles should be wiped with lint-free wipers dipped in an appropriate solvent (e.g., ethyl acetate), and the barrel should be cleaned by drawing solvent through the needle and out the top with a vacuum.

(2) Preferred Volume Range

Injection of 1-3 µl samples from a 10 µl syringe is not recommended because of the large increase in error probability resulting from these small volumes. For example, a typical absolute injection error of 0.2 µl in a 1.0 µl injection would produce a $\frac{0.2}{1.0} \times 100 = 20\%$ relative error, while the same 0.2 µl error in a 5 µl injection volume reduces the relative error to a tolerable 4% level. The analyst is strongly urged to inject volumes between 3 and 8 µl (5-8 µl optimum) from a 10 µl syringe for analyses with electron capture detectors. A syringe filled close to capacity is more difficult to manipulate. Proper dilution of standards and samples will provide on-scale peaks upon injection of optimum volumes. Standard and sample solutions are prepared so that peaks of approximately the same area are produced (Subsection 5O). With use of proper techniques, a capable analyst should be able to reproduce a series of 3-8 µl injections to within 1-5% of average peak area or height when response is ca 1/2 fsd.

The preceding paragraph describes the conventional wisdom concerning normal use of a common 10 µl GC syringe. Data have been presented, however, leading to recommended volumes between 2-4 µl. Below 2 µl, the error of injection increased above the ±4-5% range. Reproducibility decreased for samples greater than 4 µl, supposedly due to the difficulty in quantitatively transferring the total volume from the syringe because the piston sealed poorly and allowed the liquid to be forced back or leak through the back of the syringe (52).

(3) Injection Solvent

The choice of injection solvent has been shown to affect quantitation of polar organophosphorus pesticides. Hexane solution aliquots containing 4 ng of dimethoate and 8 ng of β-phosphamidon gave GC peak heights only 64% and 43%, respectively, of those from corresponding aliquots of acetone solutions. The low values with hexane apparently were caused by adsorption of the compounds in the syringe needle (53). This error was overcome by using acetone as the flushing solvent in the solvent flush injection technique [Subsection (1) above] or, preferably, using acetone to prepare all standard and sample GC solutions. Since this solvent effect is probably a general occurrence in the analysis of polar pesticides and metabolites, careful consideration should always be given to the choice of an appropriate GC solvent. Other workers have also recognized that the injection solvent can affect the precision and accuracy of GC analyses (54).

d. Capillary Columns

An inlet system for use with glass capillary columns in trace analysis has been described by Spencer (55). The system has a combination splitter and splitless design, the latter being most useful for trace pesticide analysis. In the splitless mode, microliter samples can be directly injected and the inlet backflushed to purge residual solvent from the vaporization chamber after the sample has entered the column. Relatively large samples can be injected without overloading the system and causing band spreading.

5K ERRATIC BASELINES

If all modules of the GC system are functioning properly, baseline noise should be below 1% fsd. When noise exceeds this level with the electron capture detector, all analytical work should be suspended until the cause is isolated and corrections made. A poorly regulated current supply or column liquid phase bleed can cause an erratic baseline. The slightest leak anywhere in the flow system may permit the entry of air and can be another cause of a noisy baseline. The most common points of leakage are probably septa that are not changed often enough or loose column connections.

Currently, three types of ferrules are most highly recommended for glass column pesticide work, and the choice appears to be mostly a matter of personal preference. Teflon, graphite, and Vespel polyimide-graphite-combination ferrules are all available in one piece design for use without a metal back ferrule or O-rings. Reducing ferrules for use without metal reducing unions and capillary ferrules are also available. Temperature limits are 250°C for Teflon, 450°C for graphite, and 350°C for Vespel. The three types of ferrules are reuseable at least several times if carefully removed from old columns.

Overtightening of ferrules causes deformation and limits the possibility of reuse. Expensive and valuable glass columns can also be broken. Undertightening of ferrules causes leaking and possibly allows the column to be pushed out and broken when it is subjected to increased back pressure. Most analysts eventually develop a feel for the correct ferrule torque. A typical procedure is to tighten the Swagelok fitting until finger tight and then a 1/4 to 1/2 turn with a wrench until leak tight. More precise tightening without column breakage is facilitated by use of a commercial torque wrench (available from, e.g., Supelco) that allows only the correct amount of force to be applied to each type of ferrule before slipping. The need for further tightening of connections should be checked by opening the oven after the initial overnight heating period of the column.

The column should be pushed into the fitting and then pulled back 1/16 inch before the ferrule is tightened. Because of different construction materials, the instructions supplied with each type of ferrule should be consulted before applying torque to obtain a leak-free connection. The threads on the instrument must be in good condition to allow the nut to be properly tightened, so a rethread device should be periodically used to clean the injector and detector fitting threads. Leaking ferrules are located by use of a liquid leak detector around the top of the connecting nuts or by monitoring pressure readings of the head pressure gauge.

When temperature programming is employed to facilitate complex separations, dual column GC operation will compensate for the baseline of the analytical column. The dual columns contain the same liquid phase but need not be the same length. To set up the desired baseline, the recorder and detector are first zeroed. The columns are then heated to the upper temperature limit of the program where the bleed from the columns will be greatest. The resultant baseline is adjusted to the desired baseline by varying the flow rate of the balancing column. Another approach for "high bleed" analytical columns is to place a short, "low bleed" scrubber column (e.g., a low loaded silicone) at the analytical column exit.

5L RECOMMENDED GC COLUMNS FOR PESTICIDE ANALYSIS

a. Column Selection

A number of important factors must be considered in the choice of a column or combination of columns most suitable for a particular laboratory. Some of these factors are the following:

(1) The selected columns should be capable of separating the largest number of pesticides of interest with a minimum number of overlapping peaks. For example, 10% DC-200 or 3% OV-1 non-polar methyl silicone columns are of limited value to the analyst determining the more common chlorinated insecticides in environmental or animal samples. Partially or completely overlapping peaks are obtained for several pesticides generally detected in these sample substrates, e.g., _p,p_'-DDE and dieldrin _o,p_'-DDT and _p,p_'-DDD, and the isomers of BHC (Figure 5-X, part A).

(2) A high efficiency column is desirable if injected extracts contain extraneous materials and detection of low pesticide concentrations is required. This will provide sharper separation between the peaks of interest and extraneous peaks of biological origin.

(3) Retention time or elution speed may be a primary consideration if the analyst is concerned only with quantitation of certain, specific pesticides. For example, in a project where the sole interest is to routinely determine residues of a late-eluting pesticide such as methoxychlor, the column selection and operating parameters would be tailored to elute methoxychlor in a minimal time period consistent with its separation from any extraneous peaks.

(4) Pairs of working columns should be selected to be of dissimilar polarity and therefore provide different elution patterns ("fingerprints") (Subsection 5N).

(5) Shorter columns may be adequate for chromatography of certain, late-eluting pesticides, but for multiresidue analysis of unknown samples, a 6-foot column is recommended to obtain optimum efficiency and peak resolution.

b. Phases Used in the EPA Laboratory Network

After a careful comparative study of many GC columns with the above factors in mind, the four liquid phases listed in Table 5-5 were chosen as working and confirmatory columns for the routine analysis of organochlorine insecticides in human tissues. These columns will efficiently separate the principal compounds of interest (DDT, DDD, DDE, BHC isomers, heptachlor epoxide, and dieldrin) in a reasonable time, have low bleed, and give long service when properly prepared, used, and maintained, as described in Section 4 of this Manual. They have also proven to be excellent columns for general use in the determination of many pesticide classes in various substrates. The SE-30/OV-210 and OV-210 columns are especially recommended for separation of organophosphorus pesticides to be detected with the FPD.

Each of these phases has its own peak elution pattern for the compounds of a given mixture. An efficient column of the mixed phase OV-17 (phenyl methyl silicone) with QF-1 or OV-210 (trifluoro propyl methyl silicone) separates all usual tissue peaks completely except for a ca 75% separation between p,p'-DDE and dieldrin. Higher load mixtures must be operated at very high temperature and carrier gas flow velocity to avoid slow elution and are not recommended. The SE-30 methyl silicone/OV-210 column gives no separation between lindane and β-BHC but good separation between dieldrin and p,p'-DDE on an efficient column.

The single phase OV-210 gives a full separation of the common BHC isomers, but only fair separation between the compound pairs of heptachlor,

Table 5-5

RECOMMENDED LIQUID PHASES FOR PESTICIDE ANALYSIS

Phase	1.5% OV-17/ */ 1.95% OV-210	4% SE-30/ */ 6% OV-210	5% OV-210	3% DEGS
Solid Support	Chromosorb W, H.P. or Gas-Chrom Q, 100/120 mesh	Chromosorb W, H.P. or Gas-Chrom Q, 80/100 mesh	Chromosorb W, H.P. or Gas-Chrom Q, 100/120 mesh	Gas-Chrom P 80/100 mesh
Approx. Operating T, °C	200	200	180	195
Approx. Flow Rate, ml/min.	50-70	70-90	45-60	70-90

*/ Individual liquid phases premixed prior to coating on silanized support

epoxide/*p,p*'-DDE and *p,p*'-DDD/*p,p*'-DDT. The single polyester phase DEGS gives excellent separations of BHC isomers, complete peak separations of all compounds usually in tissues, and an unusual peak sequence (β-BHC after *o,p*'-DDT and *p,p*'-DDT before *p,p*'-DDD) that makes it useful for confirmation of peak identities. The DEGS column, however, bleeds and degrades easily and has a relatively short column life. It is, therefore, not recommended as a routine working column, but only as a special purpose identification tool. An excellent pairing of columns for analysis and confirmation of residues in samples containing OCl pesticides and PCBs is either of the mixed phases in Table 5-5 and the OV-210 column. Unfortunately, pairing these columns entails GC runs at 200 and 180°C and necessitates either two gas chromatographs or one instrument with a change of column temperature and a rerun of sample extracts and standards. For example, 1.5% OV-17/1.95% OV-210 separates oxychlordane from Aroclor 1254, while OV-210 resolves Aroclor 1254 from *p,p*'-DDT, heptachlor epoxide and trans-nonachlor. This pair is, therefore, an excellent choice for analysis of samples containing oxychlordane and *p,p*'-DDT, among other OCl insecticides, plus PCBs.

Chromatograms of standard chlorinated pesticide mixtures on these columns and a single phase nonpolar DC-200 column are shown in Figure 5-X. Relative retention times of over 60 chlorinated and phosphate pesticides on OV-17/QF-1*, SE-30/OV-210, and OV-210 columns between 170 and 204°C are listed in Subsection 4,A,(6) of the EPA PAM. Use of temperatures other than those listed in the tables in the EPA PAM (preferably the temperatures indicated with an arrow) is not recommended because of the greater difficulty in comparing experimental RRT values to the tables.

Carbowax 20M modified supports (Section 4J) have been used successfully for pesticide separations, either directly or after coating with a liquid phase. The columns have been used with electron capture, Hall, and N-P detectors. Retention data and chromatograms are presented for a variety of pesticide classes in Sections 4,A,(7); 4,C,(5); 4,D; and 12,A of the EPA PAM.

c. Other Pesticide Columns

Many other phases besides those recommended in Subsection b are available commercially. Some of these may be entirely satisfactory for residue analysis while others are outdated or unsatisfactory for the task. As suggested in Subsection a, a wide range of factors must be considered in making the column selection, and a column or columns wholly suitable for one laboratory may be completely unsuited to the typical work of another.

In the early years of GC analysis, only single, nonpolar phases were utilized for the separation of the nonpolar pesticides then important,

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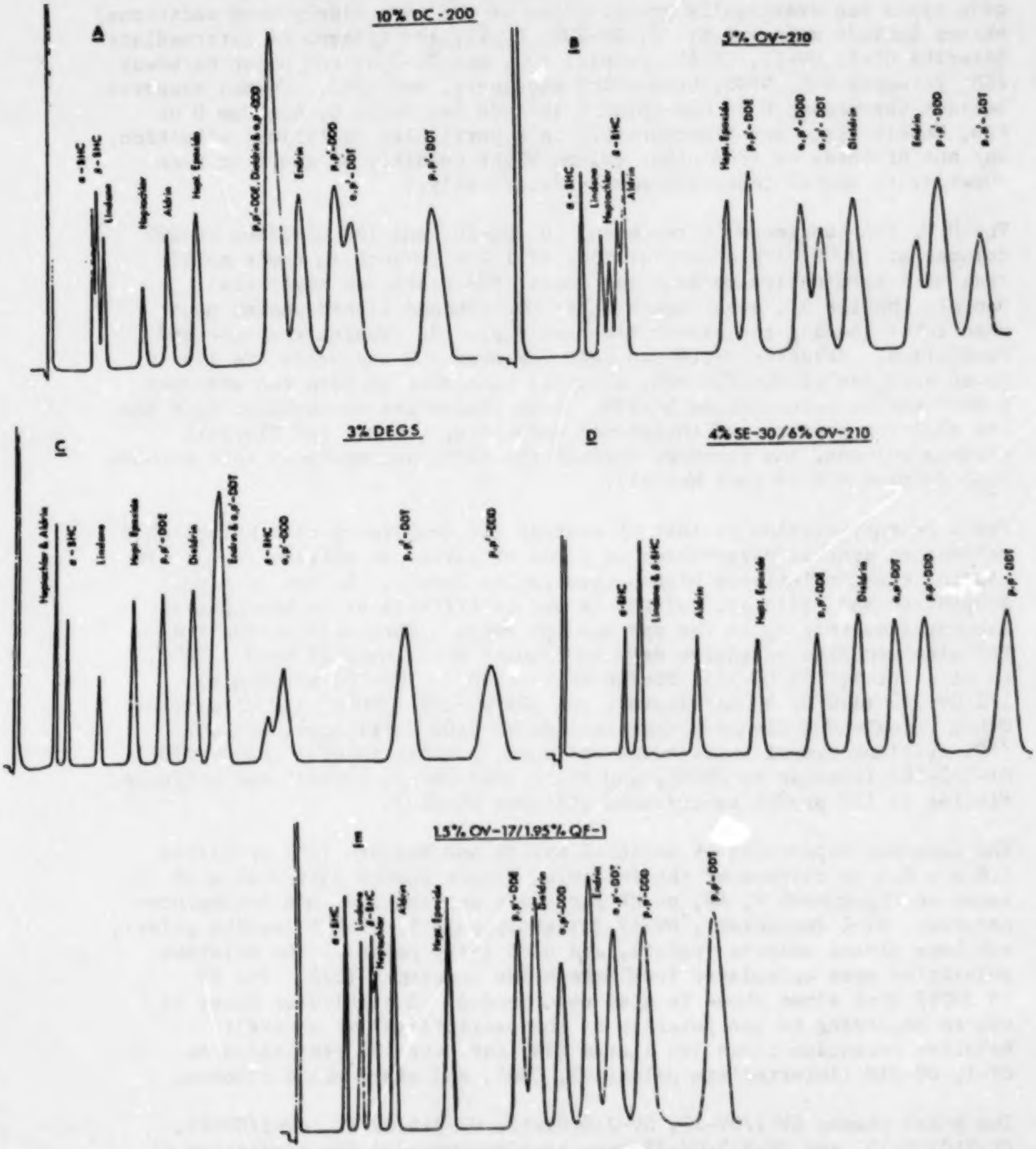
*equivalent to OV-210

Figure 5-X. Peak elution patterns of 13 pesticides on five columns.



and a survey of the literature indicates that these are still widely used today. However, mixed phase columns that combine polar and nonpolar liquids in varying degrees and newer single phases with varying polarities (the OV series) have become increasingly important as the range of pesticide types has drastically grown. Some of the more widely used additional phases include nonpolar SE-30, DC-200, DC-11, and Apiezon L; intermediate polarity QF-1, OV-17, XE-60, Reoplex 400, and DC-550; and polar Carbowax 20M, Versamid 900, NPGS, butanediol succinate, and NPGA. Common supports besides Chromosorb W or Gas-Chrom P include Gas Chrom Q, Anakrom Q or ABS, Supelcoport, and Diatoport S. In a particular analytical situation, any one of these or some other column might possibly be equal or even superior to one of those recommended previously.

The U.S. FDA continues to recommend 10% DC-200 and 15% QF-1/10% DC-200 columns at 200°C with a carrier flow of 120 ml/minute in their multiresidue determinative methods for foods (FDA Pesticide Analytical Manual, Chapter 3), even though other lower-bleed liquid phases used with lower loading and slower flow rates provide greater response and resolution. Relative retention data for over 300 compounds are listed in an appendix of the FDA PAM, arranged according to both FDA standard number and to retention on DC-200. Also listed are sensitivity data for the electron capture and thermionic detectors, eluates for Florisil cleanup columns, and recovery through FDA fatty and nonfatty food methods (see Section 9Ab of this Manual).

FDA's primary mission is that of testing for compliance with established tolerance, generally expressed in terms of parts per million (ppm). In view of these relatively high concentration levels, the use of highly responsive and efficient columns is not as critical as in the case of laboratories testing in the ppb and ppt range. Chapter 3 of the FDA PAM also contains extensive data on columns containing 2% DEGS (200°C, 60 ml/minute), 15% QF-1/5% DC-710 (2:1) (200°C, 100-200 ml/minute), 15% OV-210 (190°C, 80 ml/minute), and 10% OV-101 (200°C, 120 ml/minute). Other recommended liquid phases include SP-2100 (silicone), SP-2401 (50% trifluoropropyl substituted silicone, similar to QF-1 and OV-210), HI-EFF-1BP (similar to DEGS), and OV-11 (35% phenyl substituted silicone, similar to 50% phenyl substituted silicone DC-710).

The Canadian Department of National Health and Welfare (56) specifies 1.8 m x 6.4 mm columns of the following single phases coated at a 3% level on Chromosorb W, AW, or HP for their multiresidue monitoring procedures: OV-1 (nonpolar), OV-17 (slightly polar), OV-225 (medium polar), ethylene glycol adipate (polar), and DEGS (very polar). The relative polarities were calculated from McReynolds constants (57). The 4% SE-30/6% QF-1 mixed phase is also recommended. A particular phase is chosen according to the polarity of the pesticide(s) of interest. Relative retention times are listed (58) for over 100 pesticides on OV-1, OV-210 (intermediate polarity), DEGS, and mixed phase columns.

The mixed phases OV-1/OV-17, OV-210/OV-17, OV-225/OV-17, OV-1/OV-25, OV-210/OV-25, and OV-225/OV-25 have been recommended for separation of organochlorine insecticides, and tables of relative retentions were given for 14 compounds (59). A column packed with three silicone stationary phases, namely 2.5% OV-11 + 1% QF-1 + 0.5% XE-60 (phases premixed and pan coated on Chromosorb W HP), was shown to resolve a 14-component OCl insecticide mixture in less than 19 minutes; retention data were compared to other common pesticide columns (60). Other extensive compilations of pesticide relative retention data appear in References (61-63).

Comparison of the separating characteristics of different GC phases and selection of new, higher purity, and more stable phases to replace older ones are facilitated by tabulations of McReynolds Constants. These data rate liquid phases according to polarity and selectivity and allow prediction of similarities and differences in the ability of different columns to produce a given separation (64).

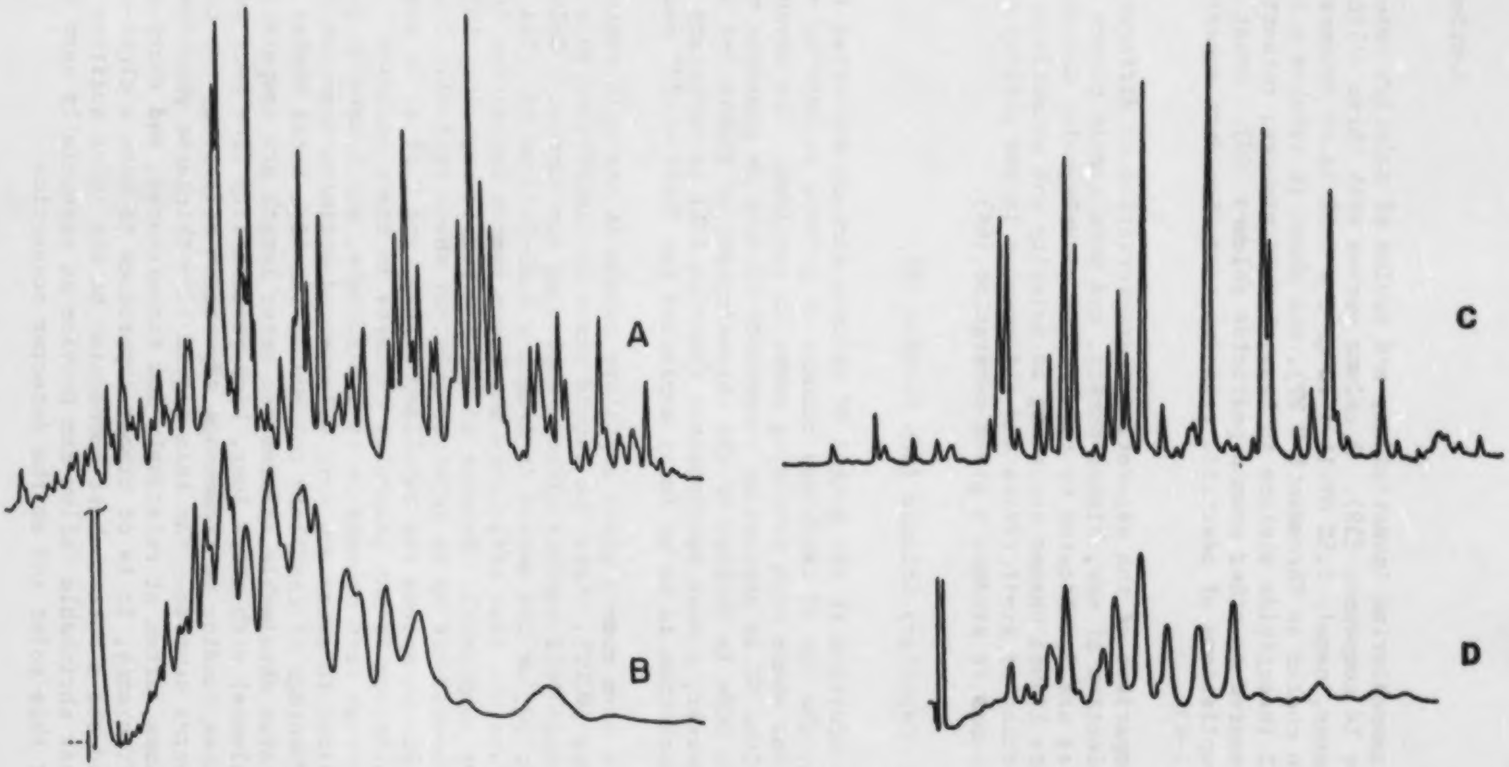
d. Capillary Columns (see Section 4M)

In addition to the packed GC columns already discussed in this subsection 5L, the use of capillary columns is growing rapidly in analytical situations where high resolving power is required. The advantage of capillary column GC in separating components of complex mixtures such as toxaphene and PCBs is obvious if the chromatograms in Figure 5-Y are compared. However, a mass spectrometer (Section 10L) is necessary if this improved resolution is to be fully exploited for "real-world" samples.

The more common glass capillary column is the wall coated open tubular type (WCOT), where the liquid phase is distributed as a thin film on the inside wall surface without employing any support. Columns are generally 25, 50, or 100 meters in length x 0.25-0.75 mm id. The smallest diameter gives the best efficiency but lower sample capacities (typically 1-50 ng per component). Because of the low sample capacity, injection is often accomplished by an injector-splitter where typically 1 µl is injected, 0.01 µl enters the capillary column, and 0.99 µl is vented. Sample splitting is not generally employed in trace analyses. Carrier gas flow through such columns is ca 1 ml/minute, and a make-up gas system is required to sweep any void volumes and optimize detector flows. The major advantage of capillary columns is the high total number of theoretical plates obtainable (plates per meter length are comparable with packed columns) with these long, high permeability (low back pressure), open tubes, leading to tremendous separation efficiency for complex environmental samples. The thin liquid film thickness provides fast analysis times, often at relatively low temperatures, and sharp peaks. To maintain efficiency, it is of utmost importance to have a clean-cut, blunt column end and a butt-to-butt connection to the inlet splitter tip assembly. Heat shrinkable Teflon can provide an essentially zero dead volume seal at this point and at the detector connection.

Figure 5-Y

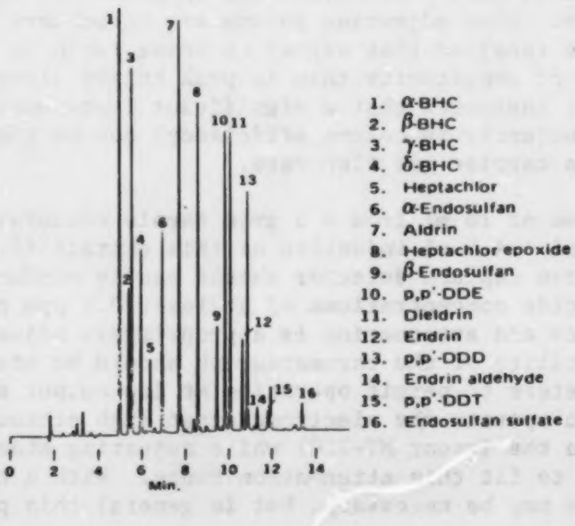
Comparison of capillary column and packed column gas chromatographic separation of toxaphene and Aroclor 1254. (A) Toxaphene on a glass SCOT column, 60 m x 0.5 mm id; (B) Toxaphene on a 3% OV-1 packed column, 1.8 m x 4-mm id; (C) Aroclor 1254 on same SCOT column as A; (D) Aroclor 1254 on a 1.5% OV-17/1.95% OV-210 packed column, 1.8 m x 4-mm id. (A) and (C) are total ion current chromatograms.



Also used are support-coated open tubular (SCOT) columns, where a layer of support (e.g., Celite) is adsorbed on the tubing wall and a liquid phase is adsorbed on the support. SCOT columns have increased capacity, wider tubing (ca 0.02 inches), and faster flow rates (4-10 ml/minute), and dead-volume connections are less critical than in a WCOT column. Sample spliting is often used but not required (sample normally <0.5 µl). Capillary columns are expensive and require good technique and instrumentation, but they are invaluable for separations requiring a large number of theoretical plates. Figure 5-Z shows a separation of 30 pg levels of chlorinated insecticide standards on a commercially coated glass capillary column (Supelco).

See Reference (65) for a review of capillary GC. Capillary columns have been mostly used in pesticide analysis for the GC-MS identification of PCBs and chlorinated pesticides (Section 10L). Retention data were reported for 60 organophosphorus pesticides and for 27 chloro-, bromo-, and nitrophenols on SE-30 capillary columns (36). The combination of an OV-101 capillary column with a pulsed mode EC detector was evaluated for quantitation of lindane, and a minimum detectable amount of 1 pg and linear response to 2.4 ng were found (66).

Figure 5-Z. Capillary column chromatogram of OCl pesticides. Column: SE-54, 30 m. Column temperature: 2 minutes initial hold, 200°C to 270°C @ 8°C/minute, final hold @ 270°C. Linear velocity: 42 cm/second, hydrogen. Detector: electron capture. Attenuation: 128. Range: 1. Sample size: 0.1 µl. Split ratio: 67:1. Sample weight: 30 pg each.



Sixty meter wall coated SE-30 columns have been used for the routine analysis of low- and sub-picogram levels of organochlorine pesticides in river and drinking water. The high resolution allowed analysis of extracts without prior column liquid chromatography cleanup. Over a three month period, 60 mixed standards and 220 sample extracts were injected into one column without a loss in column efficiency (67). Organophosphorus pesticide and metabolite residues in spinach extracts were separated on a 25 m x 0.3 mm column with diethylene glycoladipate (68). s-Triazine residue mixtures in environmental samples were separated and determined more successfully on a Carbowax 20M capillary column than on packed columns. The detection limit was 50-70 pg with an alkali flame ionization detector (69).

5M SENSITIVITY OF THE GC SYSTEM

For analysis of pesticides in environmental media, concentration of residues is commonly in the ppb or ppt range. High sensitivity of GC detection is, therefore, an obvious requirement. This is not usually so important a factor in a laboratory primarily oriented to enforcement of statutory tolerance levels in agricultural commodities or foodstuffs, since tolerance levels are usually set in the ppm range. For analyses of environmental media, the various electrical, gas flow, and temperature parameters must be optimized to produce a peak at least 20-50% full scale deflection (fsd) (with minimal baseline noise) from injection of 50 pg aldrin on one of the four recommended columns (Subsection 5L) connected to an electron capture detector. Other sensitivities (1/2 fsd) should be approximately as follows: 0.5-1.0 ng ethyl parathion for the FPD (P Model), 25 ng diazinon and 35 ng parathion for the Coulson conductivity detector (N Mode).

The foregoing subsections survey sensitivities reported for the other pesticide GC detectors. When adjusting parameters to achieve optimum response, it should be recalled that signal to noise ratio is a more meaningful definition of sensitivity than is peak height alone. It has been found in many instances that a significant improvement in sensitivity (and concurrently in column efficiency) can be achieved by simply lowering the carrier gas flow rate.

A sample extract volume of 10 ml from a 5 gram sample contains the tissue equivalent of 0.5 mg/μl. A 5 μl injection of this extract (2.5 mg of sample) into an electron capture detector should easily produce quantifiable peaks at pesticide concentrations of at least 0.1 ppm provided sensitivity is adequate and attenuation is appropriately adjusted. The high sensitivity capability of the chromatograph should be utilized by optimization of parameters to permit operation at low output attenuation. It is poor practice to operate the electrometer at high attenuations (10 x 32 or 10 x 64 on the Tracor MT-220) while adjusting standard and sample concentrations to fit this attenuation range. With a new detector foil, high attenuation may be necessary, but in general this practice, although giving chromatograms with a stable baseline, requires injections of relatively high sample concentrations to produce quantifiable peaks. This leads to more rapid contamination of the column and detector than would result from injection of less sample material, a consideration that is particularly important when injecting the 15% ether-petroleum ether Florisil column eluate from a fat sample (Subsection 9A). If the instrument is functioning properly, it should be possible to have a noise level not exceeding 2% full scale at a low signal attenuation (10 x 8 or 10 x 16).

It is important to distinguish between the terms sensitivity and limit of detection. Sensitivity is the amount of compound necessary to obtain a certain response from an instrument under a given set of instrument parameters. At maximum useable sensitivity, the response (e.g., peak height) for the compound should be at least twice the response value of the noise (70). Sensitivity can be expressed as the absolute amount of compound providing the defined response or in relative terms, such as peak height or area for a given weight of compound. Limit of detection is the concentration of pesticide above which a given sample of material can be said, with a high degree of confidence, to contain the chemical being analyzed by a definite, complete analytical procedure (71). The value depends upon the pesticide and the substrate and is expressed in relative units such as ppm or ppb (see also Section 5B).

5N QUALITATIVE ANALYSIS

In analyzing a sample extract, the first step, after appropriate cleanup and concentration, is to run a preliminary chromatogram. Assuming the chromatography system is operating under the type of control already discussed (e.g., the actual column temperature is known from the RRT_{A} p,p'-DDT), relative retention data can be related to tables [EPA PAM, Subsection 4,A,(6)] for the particular column and temperature to make tentative peak identifications. If data indicate one or more probable pesticide peaks, proper standard mixtures are selected and quantitation is carried out as described in Subsection 5O. Confirmation of peak identity is obtained by chromatography on alternate columns and/or an alternate selective detector, or by another chromatographic (e.g., TLC) or non-chromatographic procedure (Section 10). In order that some compounds are not missed, it is obviously important to allow the chromatogram to run for a sufficient time for all possible pesticide peaks to elute and be detected.

Both absolute and relative retentions have been used for qualitative analysis of pesticides. Absolute retention is the actual time between the injection of the sample and the elution of the peak. On a chromatogram, the measurement is usually made in millimeters between the injection point or the front of the solvent peak to the maximum of the peak of interest (distance x, Figure 4-C, Section 4). Conversion of retention to minutes is easily made if the chart speed is known. With detectors such as the microcoulometer that do not respond to the solvent, the injection point must be manually or electrically marked to serve as a reference point, and this must be done with accuracy.

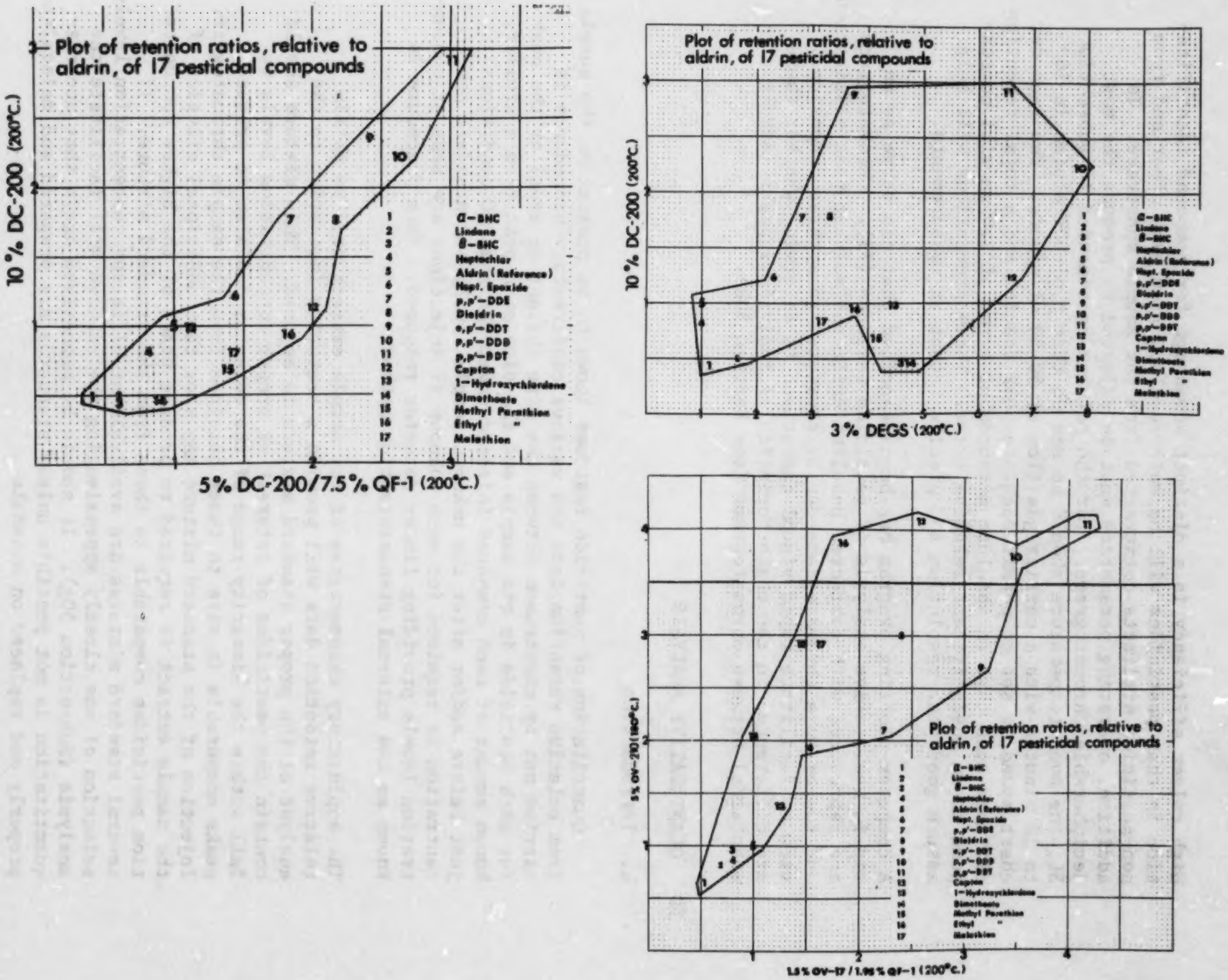
Relative retention ratio is the ratio of the absolute retention of the compound of interest to that of a reference compound, most commonly aldrin or ethyl parathion. For peaks that elute before the reference, the relative retention time will be less than 1.0; for those that elute after the reference, the relative retention time will be greater than 1.0. When reporting relative retention data, the absolute retention time and relevant instrumental parameters should be given.

The relative retention ratio is far more reproducible than the absolute retention value since only the column temperature will influence the former. Absolute retentions can vary slightly from day to day or even from hour to hour. The reference pesticide may be chromatographed just before or just after the sample, or it can be added to the sample so that its peak will appear on the same chromatogram. This latter approach is preferred if the sample is known to contain no compounds producing a peak with the same retention time as the added reference compound. In addition to relative retention, peak geometry (shape) is often an additional useful aid in comparison of sample and standard chromatograms.

Although confirmation will be treated in detail in Section 10, some comments pertaining to compound identification will be made here. The most common single factor in failure to properly identify a pesticide is the use of only one GC column. It is impossible to be sure a given column has separated all pesticides present in an unknown mixture, and if this does occur it is the result of an extreme case of good luck. Reliance on a single column is totally unacceptable and will usually lead to worthless analytical data, both qualitative and quantitative. If two columns are to be used, they should be judiciously chosen to be entirely different in their elution patterns. Complementary pairs of columns include OV-17/QF-1 with OV-210, and SE-30 with DEGS.

Elgar (72) ingeniously illustrated this point by demonstrating that when two similar columns are used and the relative retention ratios for a number of pesticidal compounds are plotted on respective axes, the points fall on a relatively straight line with little scatter in evidence. Conversely, when two dissimilar columns are used, the plotted points show a wide scatter, enhancing the probability of reliable identification. Figure 5-A,A shows the plots of three column pairs for 17 pesticidal compounds detected by electron capture. A is the plot of 10% DC-200 vs. 5% DC-200/7.5% QF-1, B is 10% DC-200 against 3% DEGS, and C is 5% OV-210 against 1.5% OV-17/1.95% QF-1. It will be observed that the RRT points plotted in A cluster to an extent that a fairly straight line is represented by the plot. Plots B and C, on the other hand, show a very wide scatter, indicating that either of these two pairs is an excellent choice for complementary columns.

Figure 5-A,A. Plots of retention ratios, relative to aldrin, of 17 pesticidal compounds on three column pairs.



High column efficiency is a distinct advantage for compound identification in that pesticides will be well resolved from each other and from non-pesticide artifacts coextracted from the sample substrate. In addition, operating parameters must be adjusted to produce the most decipherable chromatograms. For the columns recommended in Subsection 5L, the oven temperature should be set so that *p,p*'-DDT elutes in 16 to 18 minutes with a carrier gas flow of 50-80 ml/minute. The recorder chart speed is set to permit adequate peak spacing and a total retention distance such that an absolute measurement error of 1.0 mm will correspond to an insignificant relative error. These precautions will help assure good peak resolution and precise retention measurements.

A computer-plotting program has been described that can serve as an aid in qualitative analysis of pesticide residues (73). Chromatograms are reproduced with corrected baseline drift and solvent peak elimination, and two or more chromatograms can be presented in a three-dimensional view to facilitate rapid visual comparison to determine whether there are differences in the characteristics of individual peaks (sample or standards) between chromatograms (see Section 50k).

50 QUANTITATIVE ANALYSIS

a. Introduction

Quantitation of pesticide residues known to be present in the sample from relative retention data and various confirmatory procedures is carried out by comparison between the size (height or area) of the peak for each pesticide in the sample and the size of a peak from a similar, known amount of each compound injected under the same GC conditions just before and/or after the unknown sample. Only one standard concentration is required for each unknown if injections are made at concentration levels providing linear detector response. This procedure is known as the external standardization method.

The exploratory chromatogram of the sample extract used to obtain relative retention data will provide a tentative indication to the analyst of the proper standard mixture to be used. This mixture should contain the pesticides of interest at proper concentration levels to fall within the linearity range of the detector and also to produce peaks comparable in size to those obtained from the sample chromatogram. Injection of the standard mixture may show that additional dilution of the sample extract is required to produce peaks of the higher concentration pesticides comparable to those from the standard mixture. If several standard mixtures are available at different concentration levels, selection of one closely approximating the unknown will facilitate the analysis (Subsection 50g). It should be emphasized again that accurate quantitation is not possible unless standards are prepared and maintained properly and replaced on schedule.

The quantitation of complex commercial mixtures such as the pesticides chlordane and toxaphene is a difficult problem because of the inability to obtain a match between the chromatograms of real samples and standards. This problem is discussed for PCB quantitation in Section 9A,Gc. In technical chlordane, over 45 components have been identified in the electron capture gas chromatogram (Figure 5-A,B) (74). Toxaphene, which is one of the most widely used pesticides in the USA, is even more of a nightmare since it contains over 175 components (Figure 5-A,C), Both of these pesticides are more complicated than PCBs in that they contain compounds with different skeletal structures and are more susceptible to environmental and biological alteration.

Figure 5-A,B. Reconstructed total ion chromatogram of technical chlordane.

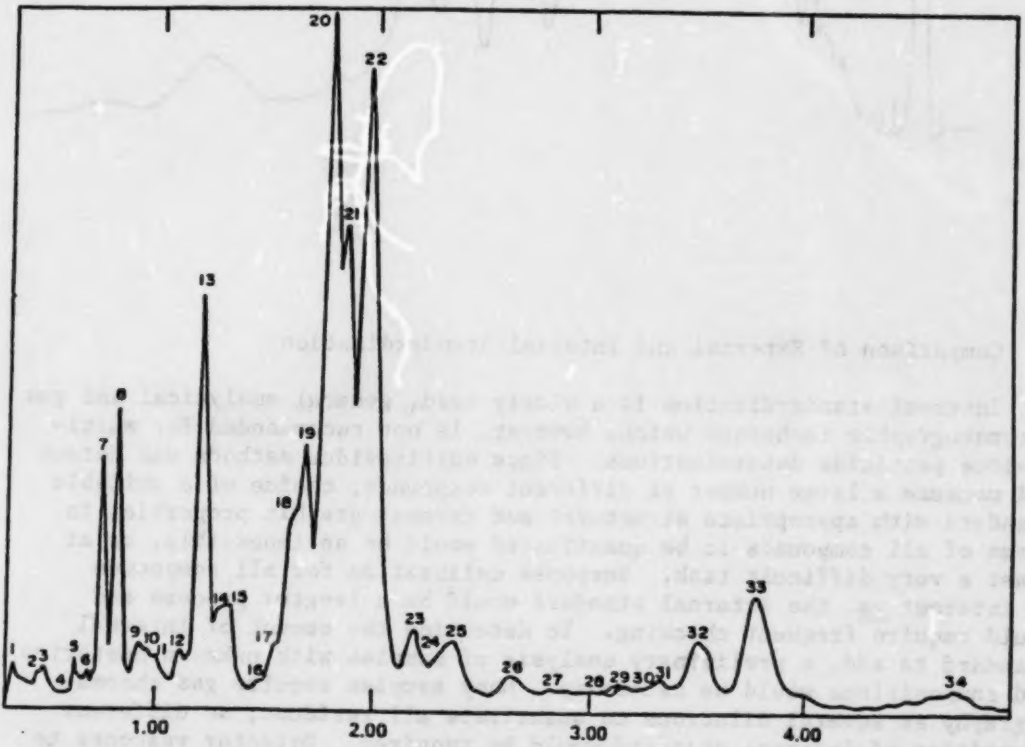
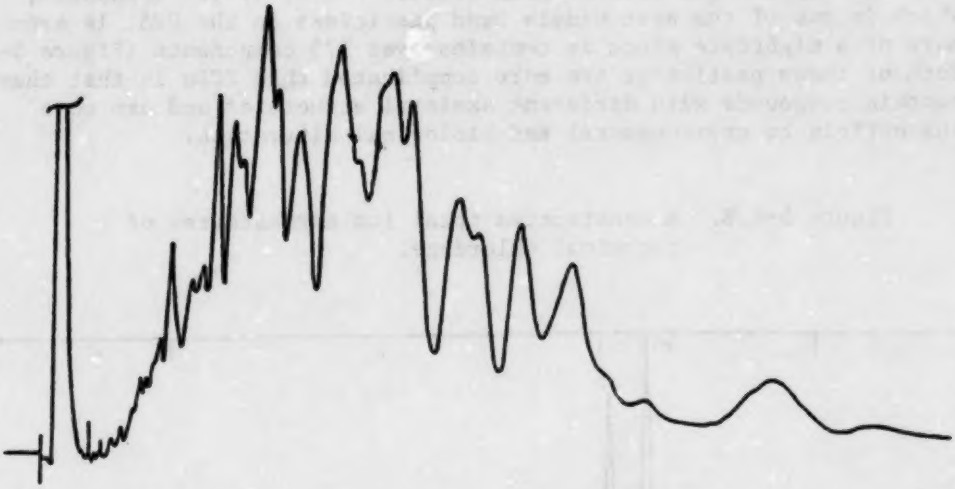


Figure 5-A,C. Electron capture gas chromatogram of toxaphene.



b. Comparison of External and Internal Standardization

Internal standardization is a widely used, general analytical and gas chromatographic technique which, however, is not recommended for multiresidue pesticide determinations. Since multiresidue methods can detect and measure a large number of different compounds, choice of a suitable standard with appropriate structural and chromatographic properties in terms of all compounds to be quantitated would be an impossible, or at least a very difficult task. Response calibration for all compounds of interest vs. the internal standard would be a lengthy process and would require frequent checking. To determine the amount of internal standard to add, a preliminary analysis of samples with unknown histories and compositions would be necessary. Many samples require gas chromatography at several dilutions to quantitate all residues, so different quantities of internal standard would be required. Detector response to sample coextractives further complicates the choice of an internal standard. These and other disadvantages dictate against the use of internal standardization except in special cases, such as in the determination of residues of one or a small, definite group of pesticides.

External standardization has the advantage that calculations are based on a comparison of the same compound in the standard solution and in the unknown, and no response or correction factor is required. Accuracy and precision depend upon the ability to inject exact amounts of samples and standards reproducibly, having all instrumental parameters under tight control so that data are comparable from run to run and determinations are conducted within the linear concentration range.

A recent study (52) concluded that generally unrecognized systematic errors were inherent in the accepted procedures of both external (direct) and internal standardization GC. For example, it was found necessary to consider both the volume injected and the concentration of the standards in the direct method; plotting peak area vs. quantity (gram, mole) is not sufficient unless the concentration is stated and the volume is kept constant. The internal standard method was found to be not necessarily independent of the volume injected, concentration of standard, or the effects of temperature and gas flow on instrumental sensitivity. The relevance of these conclusions to pesticide analysis has not been studied, and the procedures recommended in this section are based on the best current practice of experienced residue analysts.

c. Calculation Procedure

The calculation method for any GC analysis where an unknown peak is compared to a peak resulting from injection of a standard of known concentration is given below. This method is equally applicable to external standardization procedures based on comparison of standard and unknown liquid chromatography peaks or thin layer chromatography spot sizes. The equations used are:

(eq. 1)

$$\begin{array}{l}\text{ng or pg of residue}\\ \text{represented by}\\ \text{sample peak}\end{array} = \frac{\text{sample peak size}}{\text{standard peak size}} \times \text{ng or pg standard injected}$$

(eq. 2)

$$\underset{\text{(ppm)}}{\text{residue concentration}} = \frac{\text{ng in sample peak}}{\text{mg sample injected}}$$

or

$$\underset{\text{(ppb)}}{\text{residue concentration}} = \frac{\text{pg in sample peak}}{\text{mg sample injected}}$$

The following is a specific example of a calculation based on typical data on a Report of Interlaboratory Check Sample (Table 2-1):

DATA:

Sample Extract Data

gms in Final Vol.	3.0
ml in Final Vol.	25
µl Injected	5.0
mg Injected	0.60
Peak Ht or Area (mm)	145
Attenuation	$10^2 \times 32$

Reference Standard Data

µl Injected	6.0
ng Injected	0.060
Peak Ht or Area (mm)	120
Attenuation	$10^2 \times 32$

CALCULATIONS:

$$\text{wt standard injected} = 6.0\ \mu\text{l} \times 0.010\ \frac{\text{ng}}{\mu\text{l}} = 0.060\ \text{ng}$$

from (eq. 1)

$$\frac{145\ \text{mm}}{120\ \text{mm}} \times 0.060\ \text{ng} = 0.072\ \text{ng in sample peak}$$

$$\text{sample extract concentration} = \frac{3.0\ \text{g}}{25\ \text{ml}} = 0.12\ \text{g/ml or mg/}\mu\text{l}$$

$$\text{weight of sample injected} = 0.12\ \frac{\text{mg}}{\mu\text{l}} \times 5.0\ \mu\text{l} = 0.60\ \text{mg}$$

(The actual chromatogram should be labeled:

3 g/25 ml x 5 µl = 0.60 mg injected)

from (eq. 2)

$$\frac{0.072\ \text{ng}}{0.60\ \text{mg}} = 0.12\ \text{ppm residue (result)}$$

Note that the analytical data indicate a constant attenuation so that sample and standard peak sizes do not have to be corrected. When calculating the sample extract concentration, careful consideration must be given to whether the final solution contains the entire original sample or whether one or more aliquots that were taken during the sample preparation procedure have to be accounted for by a dilution factor.

The results of most actual residue analyses are not corrected for the percentage recoveries determined from spiked samples analyzed along with samples, although this can be done.

Some general comments on calculations are in order in a quality control manual. All mathematical operations should be checked at least twice, whether they are done with or without an electronic calculator. It is very easy to occasionally press the wrong calculator key or not to press a key hard enough to register. If something appears wrong with the results of an analysis, the first thing the analyst should do is to check calculations, and then ask an independent person to go over them. It is not uncommon for a person to make the same simple calculation error twice. If the calculations are correct, the next most profitable action is to prepare new standard solutions and a new standard calibration curve for the determination in question.

d. Reporting of Results (see also Section 3E)

The method for reporting analytical results will often differ from laboratory to laboratory, but in general, the following should be stated:

(1) The compounds or classes of compounds being sought.

(2) Other related or important compounds of interest that were detected or found absent.

(3) The limit of detection for each pesticide, as well as its degradation products and metabolites.

(4) Recovery values and whether results were corrected for recovery.

(5) The basis for selection of the analytical procedure and any modifications of an accepted procedure.

(6) Confirmatory methods.

Results should be reported in appropriate ppm, ppb, or ppt units, and the basis for reporting should be clear, i.e., dry weight, wet weight, or fat- or extractable-lipid basis. Any drying methods should be

described. If replicates are run, the individual results, the mean, and a statistical treatment of precision (Section 2K) should be presented.

Pesticide residue analytical data are generally reported as ppm (parts per million), ppb (parts per billion), and ppt (parts per trillion). Converting these terms to weight expressions, we have

ppm = micrograms per gram or nanograms per milligram

ppb = nanograms per gram or picograms per milligram

ppt = picograms per gram

Residues in water are quite commonly expressed as micrograms per liter, which is equivalent to ppb. On rare occasions, a laboratory may choose to express a water residue result in grams per liter, but the value becomes quite cumbersome, i.e., 5 x 10^{-7} grams per liter as opposed to the more convenient 0.5 micrograms per liter.

The following is a summary of units frequently used in pesticide analyses:

μg = 10^{-6} grams

ng = 10^{-9} grams

pg = 10^{-12} grams

ml = 10^{-3} liters

μl = 10^{-6} liters

ppm = parts per million = μg/g, μg/ml, ng/mg, or pg/μg

ppb = parts per billion = μg/l, ng/g, ng/ml, or pg/mg

ppt = pg/g, pg/ml (ppt is used frequently in other books to mean "parts per thousand").

e. Detector Linearity

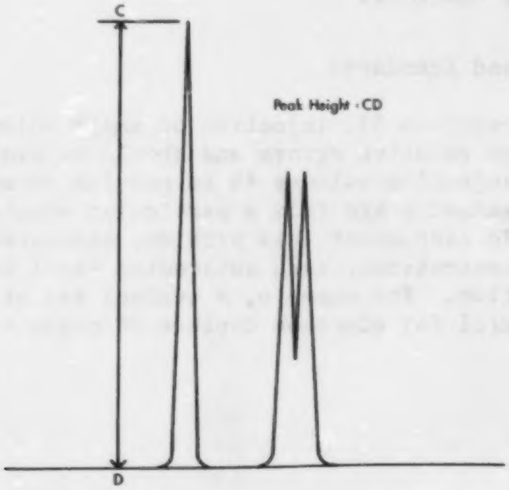
Linearity may be defined as the range of concentration over which a detector maintains a constant sensitivity. If a detector has a linearity of 10^3 and the detector sensitivity for a certain pesticide is 1 pg, the upper limit of analysis is 1 ng. If the detector sensitivity is 0.1 pg, the pesticide can be determined only up to 100 pg. Sensitivity is affected by the molecular structure and retention time for a particular pesticide under given GC conditions.

Quantitation must be performed within the linear response range of the GC detector. Each detector has its own characteristic linear range under the prevalent conditions of operation. For a given detector, the linear range varies somewhat between pesticides. For example, the isomers of BHC exhibit a more restricted EC linear range than *p,p*'-DDT. The nickel EC detector operated in the DC mode exhibits a far more restricted linear range than the tritium detector. Lindane concentrations above 600 pg may result in nonlinearity with the tritium detector, whereas the linear cut-off for this compound may occur at approximately 250 pg for the nickel detector. Section 4,A,(3),III of the EPA PAM compares typical linearity curves for various pesticides with these two detectors.

Before any attempt is made to try quantitation with a new or newly renovated detector, linearity curves should be constructed for the pesticides of interest under the prevalent operating conditions. Frequent checks should be made to insure continued operation within acceptable concentration ranges. Knowledge of the linear cut off point will preclude such error as injecting 1 ng of aldrin and expecting it to fall within the linear range of the Tracor EC detector in the DC mode.

Calibration curves are constructed by injection of serial amounts of a pesticide and calculation of the peak height of each peak. Peak height is the perpendicular distance from the peak maximum to the baseline (Figure 5-A,D, distance CD). The linear range is observed by plotting height *vs.* amount of pesticide injected.

Figure 5-A,D. Quantitation by Peak Height Method



f. Sensitivity Control

When analyses are performed in the ppb or even ppt (parts per trillion) range, electrometer attenuation is required to attain maximum sensitivity consistent with an acceptable baseline noise level. Electrometer operation at high sensitivity levels is good practice even when substrates contain high concentrations of pesticides. By serially diluting the final extract and operating at high sensitivity, the possibility of exceeding the linear range of the detector is greatly reduced and, therefore, the quantitative error possibility is reduced. Occasional instances have been observed in the EPA Quality Control Program where operators have set attenuation controls at low sensitivity and injected media containing massive concentrations of pesticides. This was readily discernable by malformed chromatographic peaks. Had attenuation been set for high sensitivity, chromatographic peaks would have gone off-scale, requiring serial dilutions of final extract to a pesticide concentration within linear boundaries.

Electron capture electrometer attenuation should be adjusted to obtain a minimum sensitivity level equivalent to a 50% fsd peak from the injection of 50 pg or less of aldrin.

There is no objection to using different instrumental attenuation settings for standards and samples provided that concentrations are within the linear detection range and checks are made to insure that the attenuator is truly linear. A sample should produce the same peak when diluted by 10 as if the original sample were run at an attenuation increased by 10. An output attenuation setting of x16 on the Tracor MT-220 chromatograph electrometer is convenient to assure operation within the range of the detector.

g. Injection Volumes and Standards

As described in Subsection 5J, injection of small volumes such as 1-3 µl can lead to large relative errors and should be avoided. A common reason for low injection volumes is to provide on-scale peaks of sample for reference against peaks from a particular single standard or standard mixture. To circumvent this problem, standards should be made up at several concentrations, each succeeding level being twice the previous concentration. For example, a typical set of three standard mixtures in pg/µl for electron capture GC might be:

	Mixture A_1	Mixture A_2	Mixture A_3
Lindane	5	10	20
Aldrin	5	10	20
Dieldrin	10	20	40
o,p'-DDT	10	20	40
p,p'-DDT	20	40	80

Injection volumes should be controlled within 3-8 µl, and sample and standard injection volumes should agree within ±25% (i.e., within 2-3 µl from a 10 µl syringe).

Working standards must, of course, include all compounds of interest to a particular laboratory. It may be necessary to run several sample extract chromatograms of various concentrations and/or injection volumes to achieve reasonable concurrence of peak size with those of the standard mixture(s) if pesticides are present in the sample at widely different levels. Peaks of standards should never be distorted. If this occurs, the injection must be repeated and the cause of distortion, if it persists, must be determined and corrected.

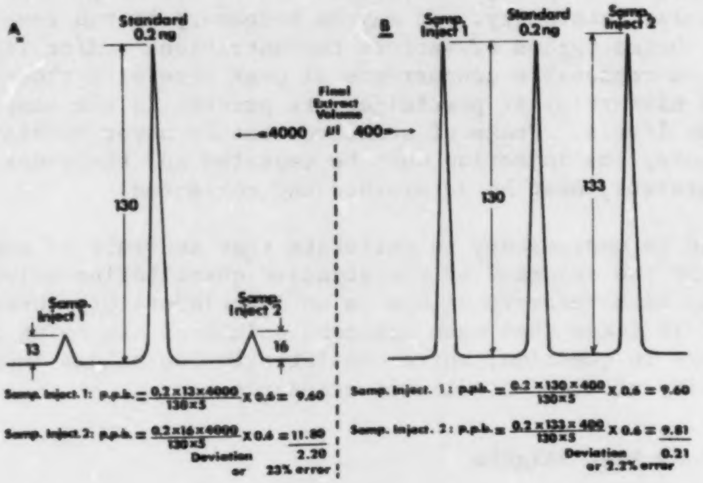
It should be unnecessary to reiterate that accuracy of analysis is limited by the accuracy of the standard quantitating solutions. Consistently high recovery values on an interlaboratory check sample strongly indicate that weak standard solutions are being used by the laboratory in question, while consistently low values indicate the probability of overconcentrated standards.

h. Optimum Peak Heights

The ideal range of peak height response is 20-60% fsd, with a minimum acceptable height of 10% fsd. Peak heights of the sample and standard should vary by no more than 10% for highest accuracy and at most by 25%. If all GC modules are operating properly and parameters are optimally set, the 10 or 20% fsd minimum peak requirement will cause no problem in terms of attainable sensitivity when standard procedures and concentration steps as given in the EPA PAM are followed. If sample peaks are too low (<10% fsd), the solution should be further concentrated or a larger amount injected. Injection of samples that are too large can cause loss of compounds in the solvent peak in some cases, e.g., HCB on an OV-17/OV-210 column.

The requirement of referencing samples against standards differing by no more than 25% in peak height causes no inconvenience when the concept of different standard concentrations (Subsection 50g) is followed. This point is important, even though one is working within the linear range of the detector, because of minor variations in GC response, primarily arising from instrumental sources and/or from small injection errors. Figure 5-A,E illustrates the potential error that is possible from a peak height variation of as little as 3 mm, when attempting to quantitate a 13 mm sample peak against a 130 mm standard peak. This is shown in (A) on the left. The total deviation results in a relative error of 23%. On the other hand, when the extract is further concentrated down to an assumed final volume of 400 µl, the height of the sample peak is increased by a factor of 10 to 130 mm as shown in (B) on the right. The same 3 mm response variation at this level will result in a final relative error of only 2.2%, a very acceptable value.

Figure 5-A,E. Illustration of potential hazard of quantitating by comparison of small sample peak against large standard peak. A 3 mm peak height shift is assumed. Initial sample size 1.67 grams; injection volume 5 µl.



i. Standard Curves

GC calibration curves prepared by injection of standards are of little direct use in residue quantitation. Such curves are not valid for extended periods of time, as is the case for other analytical methods such as spectrophotometry, and so their preparation is an unnecessary, time consuming chore. A GC standard curve constructed for an EC detector on a given day at 9 a.m. may well be worthless by the afternoon of the same day, or even the same morning, if high lipid extracts causing a rapid depression in peak response are repeatedly injected. If such peaks are referenced against a standard curve prepared when response was normal, erroneously low results will be obtained.

The proper procedure for quantitation is to intersperse standard mixture injections throughout the workday with sufficient frequency to signal the onset of response fluctuations, and quantitative referencing is made against the interspersed standards. For maximum accuracy, injection of an unknown sample would be bracketed between standard injections made immediately before and after the sample.

j. Methods of Peak Measurements

Both peak heights and peak areas are extensively used for calculations of residue amounts. The preferred method of calculation depends on the shape of the chromatographic peak. Peak height is recommended for measurement of very small peaks or tall, symmetrical, fairly narrow peaks (<10 mm at the base) that have no obscuring overlaps. These are characteristic features of most pesticide peaks from an efficient GC column, especially those that elute early. Accurate calculation of the area of such peaks would be difficult because the slightest measurement errors in the narrow width would be magnified in the subsequent area calculation. Peak area as estimated from peak height x width at half height is recommended for separated, symmetrical, and fairly wide peaks. Triangulation is used for separated, unsymmetrical peaks or peaks on a sloping baseline. Triangulation should never be used on very narrow peaks. Extreme care must be taken in the construction of inflectional tangents in all measurements. Measurements with a mm ruler containing fine division markings can be made to the nearest 0.1-0.2 mm if care and patience are exercised.

When peak heights are used, the assumption is necessarily made that operating parameters are closely controlled and retention times are very reproducible. Two consecutive injections of the same amount of compound should ideally result in two peaks with exactly the same retention time, width, and height. If chromatographic parameters (particularly column temperature) are not under strict control, the second peak may instead elute later or earlier than the first, resulting in a wider or narrower peak. However, the peak areas should be the same in both cases. For this reason, peak area or peak height x retention time is considered by many operators to be more reliable than peak height alone since slightly shifting peak positions will not be so important.

Figures 5-A,D, 5-A,F, and 5-A,G illustrate the peak height, peak height x width at half height, and triangulation measurement methods, respectively. The two right-hand peaks in Figure 5-A,D are measurable by the peak height method because their overlap does not obscure the height of either peak. Peaks on a sloping baseline but too narrow to be triangulated can be measured by the peak height method. The distance KI would be used as seen in Figure 5-A,G. Peaks can be widened by using a faster recorder chart speed. Use of the planimeter is an alternate method for measuring unsymmetrical peaks, peaks on a sloping baseline, or total area of a series of incompletely resolved peaks. Precision will be improved by tracing the peak at least twice and taking an average value.

Figure 5-A,F. Quantitation by Peak Area Method

FIGURE 5-Q QUANTITATION BY PEAK AREA METHOD

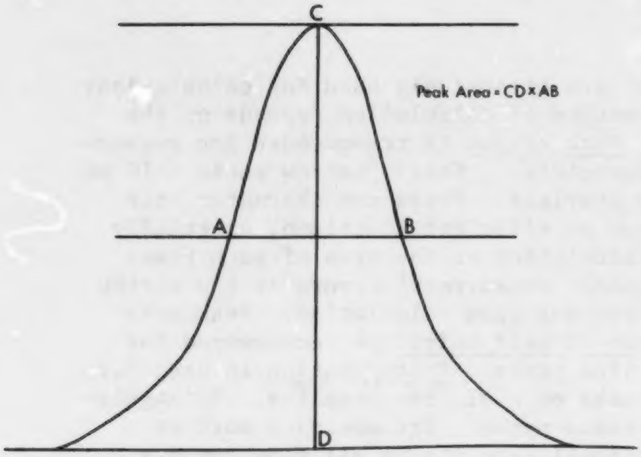
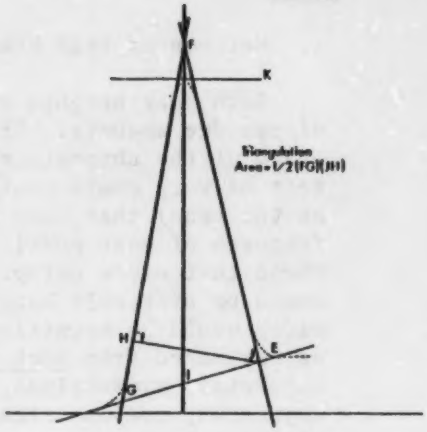

C
Peak Area = CD×AB
A
B
D

Figure 5-A,G. Quantitation by Triangulation Method

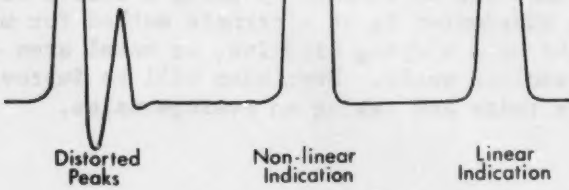
FIGURE 5-R QUANTITATION BY TRIANGULATION METHOD


F
K
Triangulation
Area = 1/2(FG)(IH)
H
E
I
G

Quantitation of peaks indicating heavy electrical overshoot (Figure 5-A,H part A) or nonlinear response (part B) will lead to unreliable quantitation. Peak overshoot is influenced by foil contamination and by improper EC detector polarizing voltage (Subsection 5Cb).

Figure 5-A,H. Examples of Gas Chromatographic Peaks

FIGURE 5-S EXAMPLES OF GAS CHROMATOGRAPHIC PEAKS


A.
B.
C.
Distorted Peaks
Non-linear Indication
Linear Indication

Automatic (disc) integration is a convenient, accurate procedure that can be used in place of manual procedures whenever baselines are steady, but it is less reliable and simple when sloping baselines or peaks with shoulders occur. This method has been mostly used for calculation of late eluting peaks and multicomponent chemicals that elute over a long period of time (Strobane, PCBs, toxaphene). In the absence of an integrator, chromatograms, especially of these complex mixtures, have been quantitated by cutting out the peaks on the recorder chart and weighing the paper. This method, although time consuming, can yield excellent results if care in cutting is taken and if the paper is uniform. Since Xerox paper is especially uniform, recorder charts can be copied and the copy cut and weighed.

Gaul (75) compared five methods for quantitation of aldrin, heptachlor epoxide, and dieldrin with a tritium electron capture gas chromatograph. The methods were disc integration, triangulation, peak height times width at half height, retention time multiplied by peak height, and peak height. No significant differences were found among the five methods in this study. The same author described methods for properly placing baselines for typical overlapping and unsymmetrical gas chromatographic peaks, and suggested procedures for quantitating multipeak chromatograms of pesticides that are mixtures of isomers, e.g., DDT, BHC, chlordane, and toxaphene. Poorly resolved peaks and sloping baselines present the greatest challenge in terms of accurate quantitation, and an experienced analyst must exercise judgment to quantitate the peaks properly. If necessary, improved resolution of peaks and flatter baselines may be sought through the use of other cleanup procedures, GC columns, or changes in operating conditions.

k. Integration and Automation

Acquisition and interpretation of data are the final steps in qualitative and quantitative analyses. How this is done can in large measure affect accuracy. The trend today is more and more towards automatic or computer assisted data acquisition and treatment. Computer acquisition is almost a necessity in mass spectrometric analyses, but it is only beginning to make in-roads into the areas of gas and liquid chromatography.

Digital computer and integration systems are available today that perform baseline detection, baseline correction, area integration, area allocation of fused peaks, and postrun calculations. They can store and retrieve GC data and visually display the chromatograms. Expansion or contraction of either the attenuation or time axis is sometims possible after the chromatogram is taken. This is very useful with a wide-range linearity detector, as it can replace multiple injections of different sample concentrations. These systems range in price from approximately $10,000 to over $100,000 per unit. When properly operated they provide very fast and accurate quantitation of chromatograms, but results may not be reliable with complex chromatograms having narrow peaks, merged peaks, variable retention times, unidentified compounds, and/or noisy and variable baselines. Despite the publication of computer methods tolerating these problems (76), the good judgment of an experienced analyst may be better in such instances. The increased use of computer systems in the future is expected as technology is improved and prices are decreased.

All systems use logic to interpret the chromatograms. The unique logic incorporated by each manufacturer in its system must be understood if the operator is to properly instruct the system how to treat the data and then properly understand the data obtained. The system can only follow the instructions by the operator, and if those instructions are incorrect, the data returned to the operator will be useless.

The capabilities of these systems and the means of handling the data differ widely, but, in general, the systems perform the same functions. The systems with built-in-printer-plotters plot the chromatogram using the digital data generated for the integration system. Generally, these digital chromatograms include peak absolute retention times and system notations to help understand the system's interpretation of the data.

All systems interpret the digital data, evaluating when the chromatograms are in the baseline or on a peak and resolving fused peaks. Once the peak is confirmed, the raw area counts and/or peak height and peak retention time are stored for later processing. Other logic and instructions from the operator are used to "draw" the baseline under these peaks and discard the area or height below the chromatographic peak. Usually these processed data are the only data stored by the system for use in later calculations. The system calibrates the peak areas and/or peak heights using the values assigned as the standard by the operator. Then all quantitation calculations are performed using the specified calculation method (area, area percent, internal standard, or external standard).

The report formats of the different systems are just as different as their logic. In general, however, the elution time, peak area and/or peak height, and the concentration of the compound are reported along with its name, if known. Naming of peaks is generally performed using the relative retention time system specified by the operator (e.g., aldrin = 1.00, p,p'-DDE = 1.00, or ethyl parathion = 1.00).

The microprocessor and minicomputer systems usually also have the capability of handling a programming language such as BASIC. The added capabilities of these systems are numerous. Postrun calculations can be tailored to the needs of the data user. Some examples of the use of programming languages include: (a) doing statistical calculations on the results of several runs; (b) modifications of report format; (c) analysis of data for a particular criterion (i.e., is the p,p'-DDT level greater than tolerance?); and (d) the handling of standards calibration, and other tasks, on automated runs. The limits of the systems with language programming are set by the capabilities of the programmer using the system.

The pitfalls of these systems are established by the experience and understanding of the operator. Accurate analyses of simple chromatograms are not difficult to obtain. Accurate analyses of complex chromatograms are quite often very difficult to obtain. One of the best ways to test the system and evaluate the usefulness of the analytical data is to closely examine the chromatograms and reports from the system. The analyst should establish if the system drew the baseline in the proper location. If not, modifications of the system's instructions are required. The same is true for the examination of the performance of all parameters in the instructions. If the instructions are not getting the desired results, the analyses must be run again after the appropriate instructions are modified. It is important to double check the system's analyses to be sure they are what is desired by the analyst.

The various approaches for automation of sample introduction in gas chromatography have been reviewed and a typical autosampler described in detail (77). Besides the advantages of automation and unattended operation for large numbers of samples, automated injection systems will normally give more precise injection volumes than most operators can achieve manually. Losses of up to 50% of aldrin and dieldrin in a commercially automated dry capsule injector were reduced by silanization of the capsules (78).

Although a reliable, automated system applicable to entire pesticide analytical procedures at common residue levels has not yet been perfected, some progress has been made on automation of residue analyses. The use of Technicon auto-analyzer modules for the automation of extraction and cleanup, followed by GC or HPLC determination, has been described and tested using alfalfa and string bean samples fortified with organophosphorus and carbamate insecticides (79). A mechanized system for 4-nitrophenol and some other phenolic pesticide metabolites in urine was reported (80). This system performs acid hydrolysis, steam distillation, and liquid chromatography separation combined with UV absorption determination, analyzing one sample every 24 minutes at 1-6 ppm levels.

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Section 6

MAINTENANCE, TROUBLESHOOTING, AND CALIBRATION OF THE GAS CHROMATOGRAPH AND DETECTOR SYSTEMS

The EPA Pesticide Analytical Manual, Appendix I, contains comments on the maintenance and repair of instruments primarily intended for laboratories that are part of the EPA or have contractural arrangements with EPA allowing them to make use of the electronic repair facility located at Research Triangle Park, NC. The Instrument Shop in RTP is equipped to handle repairs, modifications, and calibrations on various gas chromatographs, recorders, and GC detectors.

A detailed treatment of instrumental servicing and calibration is beyond the scope of this Manual. Some general comments and a few selected topics of special interest will be covered, however.

6A DAILY OPERATIONAL CONSIDERATIONS FOR GAS CHROMATOGRAPHIC INSTRUMENTATION

(a) Is the proper carrier gas connected into the system?

(1) Is the tank capable of maintaining the desired flow for an eight hour work period without going below 500 psi tank pressure?

(2) Is the tank output pressure normal (40-50 psi recommended)?

(b) What is the detector condition, temperature, flow, background current and voltage profile? Is detector set at optimum operating voltage?

(c) Is the electrometer operating properly, and is it zeroed properly? Is bucking ability adequate? What is the noise level?

(d) Has the programmer temperature remained constant? Is the temperature limit switch in a safe position to avoid accidental overheat?

(e) Does the purge system operate smoothly? Will it be used on this day's operation? When was it last checked for leaks?

(f) Is the oven damper closed? Does it function?

(g) Are all temperature set controllers functioning properly and is the voltage to the load (heaters) stable? Is the oven at proper temperature?

(h) Has the recorder been checked for proper speed, zero, gain level, dead band, ink supply, and sufficient paper? How long has it been since calibration?

(i) Are the septums, O-rings, and glass injection inserts in good condition? When were they last replaced?

(j) Is the pyrometer reading correct and are the compensator mercury batteries good? When were they replaced last?

6B CHECK LIST WHEN INSTRUMENT REPAIR IS INDICATED

A systematic check-out routine is recommended to determine whether instrumental service or repairs can be completed in-house during normal instrument off-time, or if outside help is required. Results should be written down as the check-out is completed so that full information can be transmitted for service. Serial numbers and EPA numbers of the equipment involved should be recorded as part of this information. Although the check-out presented is specifically for a Tracor MT-220 gas chromatograph, the steps are illustrative of the kind of routine that can be established for any analytical instrument.

Erratic operation of the instrument in day-to-day use is often an indication that serious trouble is imminent. Keep in mind the type of detector, column, carrier gas, and temperature range being used. Recall, or preferably, consult the instrument log to determine when the instrument was last serviced in-house (e.g., detector or column change) and if the difficulties arose directly or shortly after such service.

Check List

(a) Is there proper insulation packing in the detector compartment? Improper packing can lead to variation in signal due to ambient temperature changes.

(b) Are any wires exposed, shorted, or loose?

(c) Check proper location and readings of thermocouples and resistance thermometers; are they fully seated?

(d) Plumbing leaks should be checked for at full operating pressure before heat is applied, and again at operational temperatures.

(e) Is carrier gas pressure correct? Tank pressure should be greater than 500 psi and output pressure 40-50 psi.

(f) Are flow indicators functioning smoothly? Are they steady? Does the float go smoothly through its entire range?

(g) Are panel indicator lamps correct? Temperature programmer indicators in particular should be observed for smooth transition from heat to cool cycle.

(h) Are switches set properly, i.e., oven damper in closed position for heating modes?

(i) Does the recorder respond correctly to attenuation or input changes, bucking, zero, heat rise or cycling?

(j) Has signal to noise level increased? Refer to previous chromatograms obtained at like settings.

(k) Has the EC background profile decreased appreciably?

(l) Is the oven door secure? Also check rear access door.

(m) Are all units, such as the EC power supply, recorder, electrometer, etc., plugged into the proper power source?

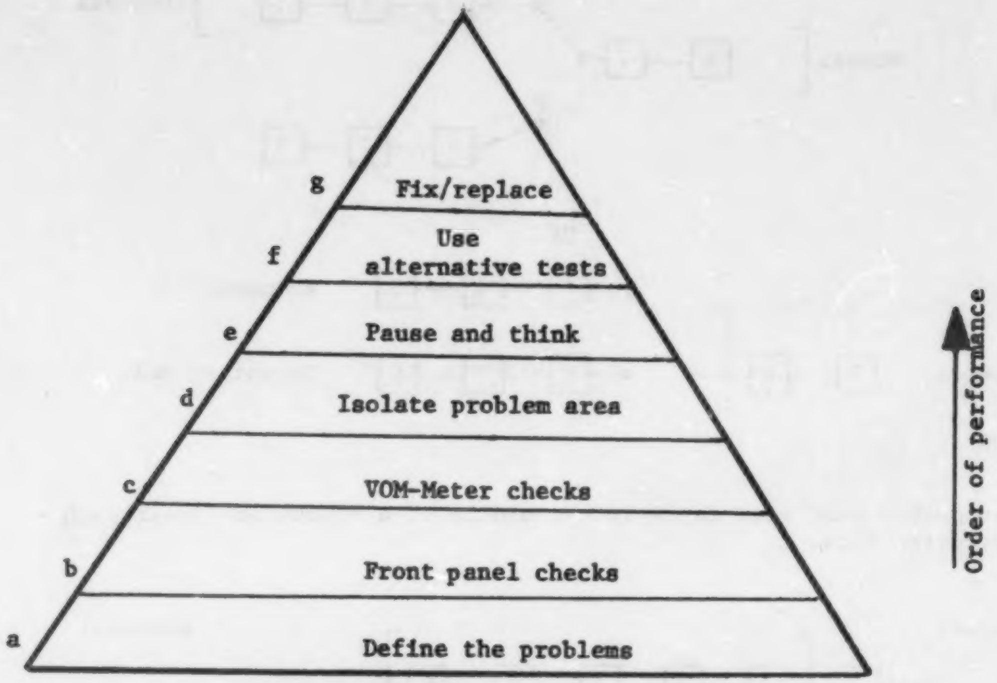
(n) Check that thermometers and thermocouples in the detector compartment are attached to the proper temperature set controllers and pyrometer points if the detector has been recently serviced.

(o) Is the column properly conditioned and is it the best one to be used in terms of stationary phase selection, efficiency, and freedom from bleed?

(p) Check color coding of circuits for accuracy. The correct color codes should always be maintained to facilitate servicing. Further tests may be recommended by service personnel to confine the problem to a particular section of the instrument. The various modules are interconnected by cables and wires, and these should be disconnected in progression from the detector toward the recorder. A volt-ohm-milliammeter with a loading factor of 20 K ohms per volt is a necessary item to have on hand when checking and servicing electronic and electrical components.

6C GENERAL APPROACH TO TROUBLESHOOTING

The following pyramid approach represents a logical, general method for instrumental troubleshooting:


g
Fix/replace
f
Use alternative tests
e
Pause and think
d
Isolate problem area
c
VOM-Meter checks
b
Front panel checks
a
Define the problems
Order of performance

(a) Write down the symptoms or difficulties observed. Diagnosis should include observations of instrument settings.

(b) Examine the problem by using the controls, meters, and lights on the instrument.

(c) Use a VOM to check voltages or resistances.

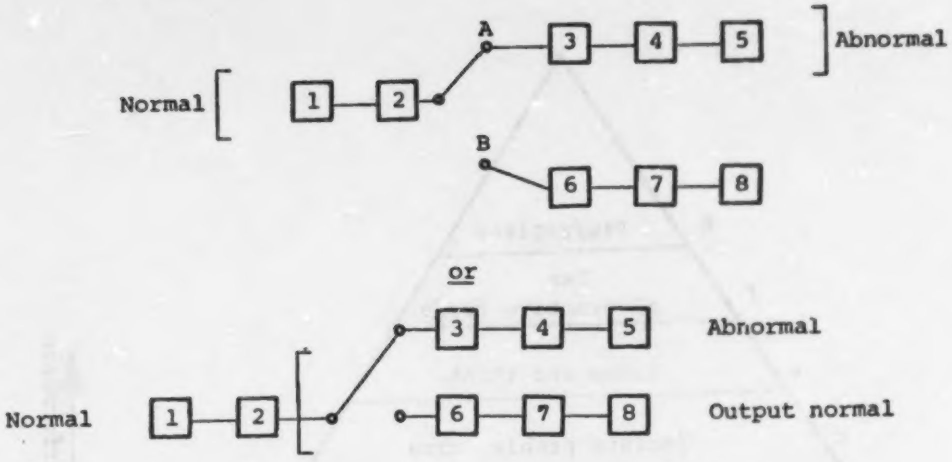
(d) Bracket the system path - electrical, mechanical, flow, and chemical. The overall system and local systems must be considered.

Isolation principles:

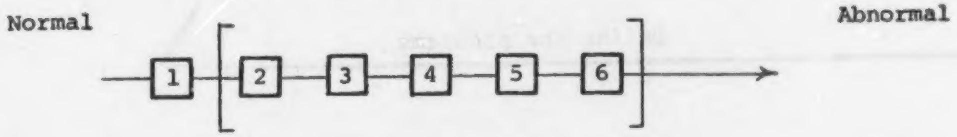
Before starting: Read the instrument manual - understand the instrument, know how to use test equipment, and understand test results.

What to do first: Use logical approach, bracket the problem to a system, bracket the area in the system, use checks to isolate faulty area.

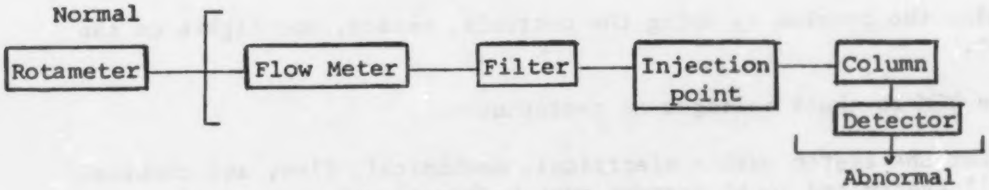
Signal paths: Use a block diagram, such as the following, and locate where in the signal path normal and abnormal output occurs.


Normal
1
2
A
3
4
5
Abnormal
B
6
7
8
or
Normal
1
2
3
4
5
Abnormal
6
7
8
Output normal

Linear path - make your check in the middle of a bracketed linear path - "Half Split" Rule.


Normal
Abnormal
1
2
3
4
5
6

Observe and measure for normal/abnormal readings


Normal
Rotameter
Flow Meter
Filter
Injection point
Column
Detector
Abnormal

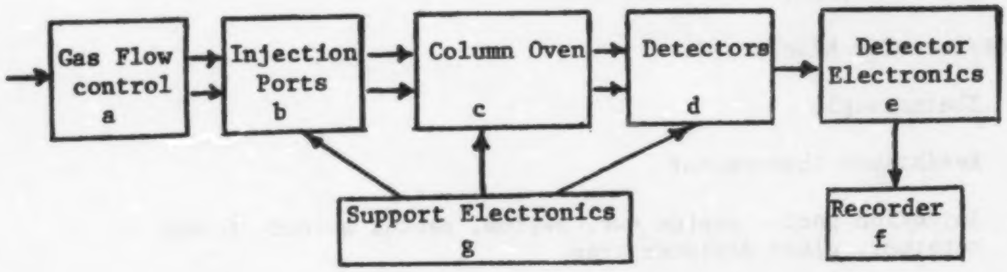
Bracket Placement
e.g., no flow: Rotameter indicates flow

(e) Pause and think - is there only one malfunction? What was changed or done recently that could cause the problem? Check with other personnel using the instrument.

(f) Verify by alternative tests.

(g) Speed, availability, and complexity determine replacement or repair procedure.

6D GAS CHROMATOGRAPH SERVICE BLOCK DIAGRAM


Gas Flow control
a
Injection Ports
b
Column Oven
c
Detectors
d
Detector Electronics
e
Recorder
f
Support Electronics
g

(a) Gas flow control: Purifies and dries the carrier gas, splits carrier gases to the columns and detectors. Controls and regulates the gas flow.

(b) Injection block: Vaporizes the sample, introduces the sample into the carrier gas stream.

(c) Column oven: Houses columns, provides a dynamic constant or programmable temperature environment.

(d) Detectors: Equalize gas temperature, detection of gas stream components, exhaust gases.

(e) Electrometer: Conditions the detector signal and attenuates the output for transmission to the recorder.

(f) Recorder: Displays an analog signal (chromatogram).

(g) Support electronics: Controls the temperature of the injection ports, column oven, detectors; indicates temperatures, indicates control voltage.

6E GAS INLET SYSTEM OF THE GAS CHROMATOGRAPH

Components:

(a) Carrier gas cylinder

(b) Two stage gas regulator

(c) Molecular sieve drying trap

(d) Copper tubing to the instrument and rotameters

(e) Rotameters

(f) Differential pressure flow controllers

(g) Tubing to the inlet

(h) Inlet/transfer block

  (1) Thermocouple

  (2) Resistance thermometer

  (3) Injection port: septum nut, septum, septum washer, insert retainer, glass demister trap.

Comments:

(a) The carrier gas cylinder used in gas chromatography is generally size "A" dry pumped nitrogen. When the EC detector is operated in the pulsed mode, the carrier gas is then 5 or 10 percent methane in argon. The carrier gas must be dry and contain less than 5 ppm O_2. Contamination of the carrier will severely affect performance.

(b) A two stage gas regulator should be used to reduce and regulate the carrier gas pressure. The first stage gauge indicates the cylinder pressure while the second stage indicates the reduced pressure to the chromatograph. A diaphragm valve on the regulator allows control of the output pressure, which should be 40-50 psi.

(c) Molecular sieve drying traps should be installed between the regulator and the chromatograph to prevent water and hydrocarbon contamination from entering the gas chromatographic flow system. Molecular sieve 13X (1/16 inch) pellets have been found satisfactory for the filter load and should be baked-out at 350°C in a nitrogen stream for four hours prior to use and capped off for storage. It is advisable to do this with every cylinder change. Occasionally the dryer body should be cleaned with hexane-acetone before reloading. It may also be necessary to flame the dryer frit to expel all contamination.

(d) Copper tubing, 1/8 inch, instrument grade is used between the filter dryer and the instrument. This tubing should be rinsed with methylene chloride and then acetone before installation. If the old tubing is used, it should be also flamed. It is also advisable to clean all Swagelok fittings before installation. Swagelok nuts should be placed on the tubing for use before being placed on the instrument. This insures proper Swage connection and reduces the possibility of damage during installation.

(e) The rotameters generally used in the carrier stream are the Brooks Sho Rate 150. They are calibrated for the pressures encountered in GC work. Charts of the calibration curves are readily available. Contamination of the rotameters may cause the float to stick. Moisture in the tube will appear as a ring around the float. The tube must be removed by loosening the hex screw at the top of the rotameter body. Do not attempt to clean the tube in the rotameter body since solvents will attack the O-rings or seals causing them to swell. It is also possible that the O-rings will absorb the solvent and bleed vapor into the system. The tube should be cleaned with hexane, acetone, and Freon MS-180. A flow meter should not be placed downstream of the flow controller but rather between the carrier gas inlet and the flow controller.

(f) The differential flow controllers (Brooks) are composed of a needle valve/seat assembly and a diaphragm, preferably Teflon, to maintain a constant flow of carrier through the column, even though the pressure drop across the column changes. The controller requires at least 25 psi for proper operation and may flutter under lower pressures. It is recommended that 40-50 psi be used to operate them properly. In programming, it is recommended that 60 psi be used to insure proper response of the system.

The needle valve/seat assembly may occasionally stick. This occurs when the needle becomes lodged in the seat. The valve must be taken apart and the needle and seat cleaned. Experience has indicated that when this occurs, a new needle valve/seat assembly is advised. The main cause of damage to the assembly is through improper operation: never close down forcibly, never open wide past the point that the float rests in the upper part of the flow tube. Since these are differential controllers, there will still be slight gas flow when the rotameter float is at zero. This is not uncommon as a flow controller is not a shut-off valve!

It is advisable to install a small frit spring-loaded filter on the outlet of the flow controller. This is a Brooks type 8501/8502 unit that will protect the controller from flashback and trap solid contaminants from the carrier gas, or filter.

(g) Copper tubing 1/8 inch od is used from the flow controller and is joined to the 1/8 inch od stainless steel tubing of the inlet assembly with 1/8 Swage to 1/8 Swage unions. It is advisable to replace these unions with a Swagelok slide valve, 1/8 inch. This will aid in checking the system for leaks up to this point and may enable operation of one column while repairs are being carried out. By closing off the slide valve, the float should drop to zero; if it does not, there is a leak up to that point. The 1/8 inch stainless steel line is welded into the inlet, which is secured by screws into the inlet heater block.

(h) The inlet block is an aluminum block with facility for inserting the inlet thermocouple and a heater cartridge. There is also an orifice for inserting a resistance thermometer. With the addition of other detectors in the head compartment, this resistance thermometer may not be used in many cases and the inlet is heated (225-230°C) by controlling voltage through a Variac voltage regulator. This inlet tube assembly has a septum retaining nut which holds the septum in place and has a small orifice for insertion of the syringe needle into the port. The septum washer (stainless steel) is placed under the septum and above the insert retainer to allow for a flat surface above and below the septum. The glass insert retainer should be turned down until it comes in contact with the glass insert and then backed off 1/8 turn. The Vykor glass insert should be removed daily and replaced with a clean one while the used insert is cleaned in the prescribed manner (Subsection 5J in Section 5).

Care should be taken to insure that the thermocouple and heater leads do not become frayed. A small amount of insulating material or glass tape should be used between metal surfaces and these wires. Avoid sharp bends close to the element.

6F PROCEDURE FOR ISOLATING PROBLEMS IN FLOW SYSTEMS OF ELECTRON CAPTURE EQUIPPED GAS CHROMATOGRAPHS

(a) Allow the column oven to cool to ambient temperature, set all rotameters to zero, and close off all oven exit ports with 1/4 inch Swagelok plugs.

(1) If the EC detector is suspect because of poor total response regardless of the amount of polarizing voltage applied to the detector, install an EC detector with a new foil.

(2) Prepare a line filter filled with 13X (1/16 inch) molecular sieve pellets and activate as previously described. Place it at the dual-stage regulator output of the carrier gas tank. The dual-stage regulator should be set to deliver 40 psi to the system.

(3) Insure that a cylinder of carrier gas which is known to be free of contaminants is being used in the system.

(4) Attach the detector purge line to the proper purge connection and set the rotameter for a flow of approximately 90 ml/minute.

(5) Adjust the detector temp-set controller to maintain a detector temperature of approximately 200°C. If a faulty temp-set is encountered, substitute a Superior type B Variac.

(6) Obtain a profile for the background current signal (BGS) by increasing the polarizing voltage in five (5) volt increments, noting on the chart the step characteristics and the maximum deflection voltage.

(7) The resulting profile should approximate the profile provided with the detector in characteristics and maximum deflection voltage although slight variations may occur. Particular attention should be paid to the step increase from 0-5 volts and from 5-10 volts. A leak or contaminant in the flow system are common causes of a poor profile (Figure 5-E in Section 5).

(b) Corrective measures for locating faults in the purge loop.

(1) Check for leaks at all fittings in the purge loop with "Snoop", beginning at the cylinder. At this point, also use "Snoop" to check for leaks on the valve control stems.

(2) Change the detector purge line to another rotameter on the purge module.

(3) Clean all lines in the purge loop or replace with new ones that have been cleaned and flamed. All tubing that is changed should be replaced with instrument grade tubing, and it is recommended that this tubing also be cleaned prior to installation.

(4) If the condition of the carrier gas was not known at the time the above tests were made, a new cylinder of carrier gas should be tested. All new cylinders should be checked for leaks at their welds and outlet valves.

(c) After obtaining a satisfactory profile with purge, place 1/4 inch Swagelok plugs on all four column inlet fittings inside the oven.

(1) Replace or remove the glass demisters in the inlets.

(2) Replace the inlet septums with new and preconditioned septums.

(3) Adjust the column #1 flowmeter to deliver approximately 90 ml/min of carrier gas. The rotameter float should rise and very shortly should drop to zero if there are no leaks in the column #1 flow system. Repeat this on the other carrier systems. If none of the floats fall to zero, the leak is usually common to all ports. The most likely area for this type of leak is at the pigtail fitting on the instrument rear, although there have been instances of such leaks occurring due to a cracked inlet block.

(d) Corrective measures for locating leaks in the carrier flow system.

(1) Open the 1/8 to 1/8 Swagelok unions on the rear of the chromatograph where the copper lines meet the stainless steel lines and cap off the copper line at this point. Upon applying carrier flow as stated in (c) (3), the float should drop to zero. If the float does not fall, the leak is in the flow controller or rotameter. It is possible to tighten slightly the flow tube in the rotameter by the hex adjustment located on the top of the rotameter housing.

(2) Check all lines with "Snoop" from the "pigtail" to the Swagelok plug and observe for leakage.

(3) Observe the rotameter float for "bounce" or rapid but slight fluctuations; this will usually indicate a faulty diaphragm. Do not attempt to repair the diaphragm but replace the total unit with a new flow controller.

(e) Install in all ports empty glass columns that have been thoroughly cleaned, taking care that they are properly seated.

(1) Allow the system to remain under carrier flow for at least 30 minutes to evacuate air introduced into the loop during installation of the columns.

(2) Follow the procedures outlined in (a) (6) and (a) (7) for each port.

(3) If an acceptable BGS profile is obtained, the flow system is free of leaks or contamination under ambient temperatures.

(4) Slowly increase the temperature of the inlet, oven, and transfer lines to their operating levels and obtain a BGS profile for all ports.

(f) Corrective measures for locating faults in the carrier flow loop.

(1) Test for septum leaks, if new septums were not used, by rapidly cooling down the septum nut with water and allowing a small quantity to remain in the septum nut depression. Observe this water-filled depression for bubbles that would indicate a leak.

(2) Insure that the columns are seated and sealed properly. It is usually advisable to tighten the columns an additional 1/4 turn after heat has been applied to the oven.

(3) Tighten the transfer line fittings, but take care not to strip the fitting threads.

(4) Inspect for a cracked block. By turning up the carrier flow to a high level, it is sometimes possible to hear the escaping gas.

(5) If the steps taken do not correct the problem, it may be assumed that there is contamination in the carrier loop.

i. Remove all fittings in the flow loop and clean or replace them.

ii. Clean the inlet and outlet ports while heated and under carrier flow with Pre-Post 1001 cleaner. Use a pistol cleaning brush.

iii. Flame all lines under carrier flow where possible.

iv. Remove and clean the flow controllers and dry thoroughly with carrier gas. Be sure to remove all moisture from the controller diaphragms.

v. Add an in-line filter loaded with 13X molecular sieve at the junction of the copper and stainless steel tubing until an acceptable BGS is obtained. If, after the addition of the in-line filters, a proper BGS is obtained, it may be assumed that the problem is in the rotameter or flow controller area. Do not operate with these filters permanently. Install a new carrier module and replace all tubing from the bulkhead fittings to the in-line filters.

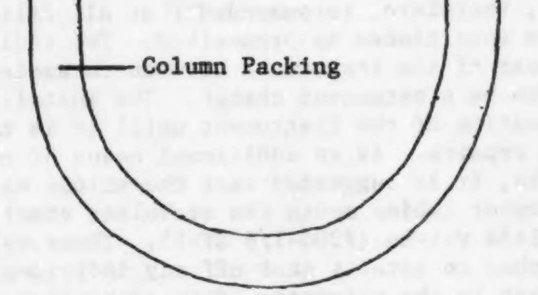
vi. Install well conditioned columns and allow the system to equilibrate. If in-line filters remain in the carrier loop, allow additional equilibration time because of the greater volume in the loop. Flow control changes will take approximately 30 minutes to equilibrate with these filters in the system. Obtain a BGS profile from all ports. Insure that the columns are not filled to the point where the packing will come in contact with the metal inlet and outlet fittings. The higher temperature at these points may cause the column packing to bleed. Slowly raise the oven temperature to 100°C and obtain a BGS profile. If it is satisfactory, raise the oven temperature to full operating temperature and obtain a BGS profile. If it is again satisfactory, the instrument should now be in full operating condition.

It has been the experience of the RTP Laboratory that in-line filters loaded with 13X molecular sieve are superior in performance to those loaded with type 5A. It is, therefore, recommended that all filters used be charged with 13X and conditioned as prescribed. The addition of in-line filters at the rear of the instrument between rotameters and column inlets is not meant to be a permanent change. The installation of these filters allows operation of the instrument until it is convenient to obtain materials to make repairs. As an additional means of rapidly checking the system for leaks, it is suggested that the unions on the instrument rear where the copper tubing meets the stainless steel tubing be replaced with Swagelok slide valves (#200-1/8 SV-6). These valves will enable the chromatographer to totally shut off any individual carrier loop so that the float in the rotameter, flow controller, and lines can be checked for leaks. These valves may also be used if repairs and operation of the instrument are to be carried on simultaneously.

6G GENERAL INFORMATION - FLOW SYSTEM

Faulty flow controllers will cause the flow to change from time to time. The controller may also exhibit short term fluctuations and may completely open or close. Care must be taken in operating flow controllers; they are never opened past the full scale indication of the rotameter or closed down in an attempt to completely shut off gas flow. Always maintain a cylinder pressure in the 40-50 psi range. Always change the carrier gas cylinder when it depletes to 500 psi cylinder pressure. Check flow through the system at the detector effluent line weekly with a bubble meter. This will indicate the proper function of the instrument and condition of the column packing to some degree. As the instrument vibrates, the columns may tend to pack down tighter causing a decrease in flow and may also affect the retention time. To check for a worn or bad flow controller diaphragm, operate the unit at 40 psi, noting a setting on the rotameter. Increase the pressure to 50 psi and note the rotameter setting. If it shows an increase of 4 or more divisions, the controller is faulty. A Brooks filter, #8501, may be used at the outlet of the flow controller to protect the system from particulate contamination and to some extent from flashback.

When installing columns in the instrument, they can be set-up as shown in the diagram following. The lower O-ring is not critical and is used simply to support the Swagelok nut. A clearance of at least 1/8 inch is recommended between the column packing and the fittings after installation is completed.



6H TEMPERATURE CONTROL AND INDICATION IN THE GAS CHROMATOGRAPH

The inlet and transfer system may be controlled by a feedback bridge type SCR controller. This controller uses a resistance thermometer and a potentiometer for control. It has been found advantageous to utilize variac control of the inlet and transfer where possible (cost- and trouble-free operation). Variac control is possible when ambient conditions are stable.

The electron capture detector temperature should always be controlled by the feedback bridge type SCR controller, as minute changes here may cause cycling or shift due to small changes in ambient temperature or improper insulation in the detector cage. There should be no detector body area exposed, as slight air currents may cause cycling or drifting baselines.

A pyrometer (0-500°C) is generally used to indicate the temperature of various thermocouples in the instrument. A switch located adjacent to the pyrometer is used to connect various thermocouples into the circuit to be monitored. The thermocouples used in the chromatograph are usually terminated in a compensator, which is a cold junction reference bridge circuit that compensates for ambient room temperature. This circuit is dependent upon thermistors in the bridge circuit and mercury (RM-12) batteries that should be checked monthly with a battery checker or whenever temperature indications appear faulty. (Usually one thermocouple is placed in the open air to rough-check the pyrometer against ambient temperature). Always be sure that battery contacts are clean.

Oven heating and control are obtained by:

(a) Two resistance wire heaters in the oven walls secured to plates attached to the walls and wired in series with a limit switch.

(b) Two thermocouples (metal sheathed). T/C is used with older type programmer units. Ribbon Resistance Thermometers (50 ohms) will be used in newer types for temperature sensing.

(c) Fan motor and squirrel cage blower assembly.

(d) Damper system.

The oven is generally a stainless steel unit insulated with micro fiber insulation cover. The design permits rapid heating and cooling dynamically to desired temperature equilibration.

The top sheathed thermocouple is used as the temperature indicating unit, and the bottom sheathed thermocouple is used for programmer control. This placement is non-critical.

The programmer contains an initial control circuit which is used mainly in isothermal control of the oven and a final control circuit activated for programming. The programmer circuitry may be used to raise the temperature at a specific rate and automatically return to a set temperature by proper use of the adjustments on the front panel. The oven temperature controller will not be activated until the fan/blower switch is turned on.

The onset of pyrometer problems is often insidious, and problems may be prevalent for some time before the operator becomes aware of them. One simple and inexpensive means of monitoring this, if the oven design will permit, is to drill a 1/8 inch hole in the oven door at a point opposite the center of the columns; insert through the hole the 8 inch stem of a dial face bimetallic thermometer (Weston model 4200, 0-250°C). While these thermometers are not highly precise, they are sufficiently accurate to provide the operator with an indication of trouble in the pyrometer network. If the dial face thermometer is reading 185°C and the pyrometer readout is 200°C, a problem is indicated not only in the column oven temperature but in the other heating modules in the instrument, i.e., injection port, detector, transfer line, etc.

6I DETECTOR AND ELECTROMETER

The detector and electrometer are integral parts of the gas chromatograph. They are connected together by BNC to BNC Teflon coaxial cables, one for the polarizing voltage and the other for the detector signal. The electrometer supplies a negative DC voltage to the detector at a regulated constant rate. The radioactive detector source is encased in a Teflon (^{3}H) or ceramic (^{63}Ni) cylinder. This cylinder, in turn, is encased in a stainless steel block which serves as a heat sink heated by a 50 or 100 watt heat cartridge. When a ^{3}H (tritium) detector is used, an adjustable limit switch in series with the heat cartridge prevents the detector from being heated above 225°C. If the temperature is allowed to exceed 225°C, excessive losses of tritium from the foil or damage to the Teflon parts will result. Such temperatures create no problems with the ^{63}Ni detector, thus a limit switch is not needed.

The electrometer input attenuator is comprised of high resistance glass resistors forming a decade stepping switch. These resistors are affected by dust, temperature, and light. They must be maintained extremely clean and never hand touched. The highest attenuation available is 5×10^{-13} amps. This is obtained at the 0.1 setting. A minimum attenuation of 5×10^{-8} amps is obtained at the 10^6 position.

The output attenuator is a binary resistance switch that enables further attenuation in a 1 to 256 range. A potentiometer in the output section of the unit adjusts zero balance of the electrometer amplifier to permit adjustment of the output voltage for zeroing the strip charter recorder. A bucking control utilizing two glass resistors for normal or high bucking is located on the electrometer rear apron. This bucking control is the coarse bucking allowing either 10^{-8} for electron capture operation or 10^{-10} for flame. A 10 turn potentiometer is located on the front panel for fine bucking control.

Internally, the electrometer contains two plug-in printed circuit boards, one for power conditioning and the other for amplification, zero, and balance. This is in addition to the input and output attenuators. It is important, due to the extreme sensitivity, to operate the electrometer with its cover correctly in place, otherwise noise will be excessive due to stray field pick up.

Electrometer Problems:

(a) Cannot zero and/or cannot buck out - possible cure:

(1) Check zero and bucking pot on front panel for continuity.

(2) Check 1 percent resistors on amplifier board for continuity.

(3) Check 2N699 transistor on amplifier board for leakage.

(4) Check 4 mfd @ 50V non-polarity capacitor on amplifier board.

(5) Check all zener diodes on power supply board.

(b) To adjust trim pots on amplifier board:

With output attenuation on 1, turn input attenuator to off and turn zero pot on front panel five turns from either extreme. Then adjust zero balance trim pot until recorder reaches zero.

Electrometer Drift Check:

(a) Set master switch to off and completely disconnect the electrometer from all test equipment.

(b) Connect recorder signal cable to the electrometer.

(c) Set input attenuator to 0.1 and output attenuator to 1.

(d) Adjust bucking potentiometer for recorder indication of 50 divisions.

(e) Set recorder to slow speed.

(f) Allow units to run in this condition until recorder does not deviate more than 5 divisions per hour.

(g) Set output attenuator from position 1 to position 256 slowly, noting pen deflection. This should not exceed 0.25 chart division through the total range.

Solid State Linearizer:

A recent innovation introduced for use with electron capture detector systems in the constant current pulsed mode is a solid state linearizer. The linearizer enables the chromatographer to operate the electron capture detector over a dynamic range of at least 20,000:1.

The linearizer requires a warm-up period of at least 12 hours after installation or after any flow interruption. Argon-5% methane is the preferred carrier gas; however, a 10,000:1 dynamic range may be obtained using high purity nitrogen.

Malfunctioning of the solid state linearizers has been observed where the recorder will suddenly go off scale and remain so until the unit is shut down for a period of time. Upon reactivation of the system, the unit appears to function normally. If this occurs, refer to the operations manual schematics and:

(1) Change R_2 and R_3 to 1K½W

(2) Change VR_2 to 7.5 or 8.2V

(3) Add 330 mfd at 10 VNP across R14

(4) Add 1 mfd at 50V in series with a 10 ohm ½W across CR5.

These changes will improve the operation of the linearizer and reduce noise to an acceptable level. It may be necessary to re-zero the unit after these changes. The Tracor service manual procedure "2" states "Remove clip lead." This is incorrect. The clip lead should be retained. In procedure "3", do not clip a shunt lead from E_4 to ground and do not adjust to zero but to 30 MV.

Printed circuit board 1700 - 504400H cannot be repaired or aligned in the field and must be returned to the factory for replacement.

All printed circuit boards in a linearizer should be removed annually and spray cleaned with Freon MS-180.

6J OBSERVATION OF PROBLEMS ON CHROMATOGRAMS

(a) Peaks return below baseline: dirty or partially depleted detector foil - clean or replace foil.

(b) Peaks have flat tops: check for proper oven and detector temperature and recorder gain control.

(c) Insufficient peak height: check for proper attenuation settings, proper amount of injection, recorder response.

(d) Tailing peak: check for proper operating temperature and gas flow.

(e) Stepping baseline (may be observed on peaks): check for dirty recorder slide wire, line voltage changes, recorder drive, recorder gain control.

(f) Noise level: check for approximately 1 division of noise @ 10 x 8 attenuation.

(g) Spikes: check external polarizing voltage unit, line noise, noisy temperature set controller, dirty system, regulation diaphragm.

(h) Rapid cycling: check oven temperature programmer, oven control thermocouple, compensation circuit, temperature set controllers, limit switches.

(i) Excessive noise in baseline: check for module noise by elimination or substitution, ground loops, recorder gain (properly set?), cable connections (coaxial), leaks in flow system.

(j) Noise with erratic spikes: check for proper carrier, clean carrier, leaks, ground loops, column bleed.

(k) Slow cycling baseline: check oven limit switch, damper operation, control thermocouple, thermocouple compensator.

6K DETECTOR BACKGROUND SIGNAL (BGS) RESPONSE

(a) Normal detector response - has good maximum signal (BGS).

(b) Abnormal detector response - has poor stepping, does not saturate at approximately 25 volts (Figure 5-E, Section 5).

Possible Problems:

(1) Moderately contaminated carrier gas.

(2) Bleeding or unconditioned column (absorbs BGS).

(3) Positive voltage on detector.

(4) Leak in system.

(5) Detector in heating cycle (wait until pyrometer stabilizes).

(6) Reversed coaxial leads from electrometer to detector.

(7) Contaminated radioactive source in detector.

(8) Contaminant flowing from previous injections (residue bleed).

(9) Dirty, bleeding, torn septum.

Troubleshooting:

(1) Leave column cold. Eliminates problems (2) and (8).

(2) Check system from tank to detector fittings. Eliminates (4).

(3) Observe voltage with VOM. Eliminates (3).

(4) Check coaxial connectors. Eliminates (6).

(5) Check detector temperature for stability. Eliminates (5).

(6) Observe standard solution injection - if peaks are not below base. Eliminates (7).

(7) Replace septums with conditioned ones. Eliminates (9).

(8) Change carrier gas tank. Eliminates (1).

6L TROUBLESHOOTING COULSON ELECTROLYTIC CONDUCTIVITY SYSTEM

(a) High background.

Symptom: Unable to zero system with bridge. Bridge attenuator x8 or below.

Probable cause:

(1) Recorder zero inaccurate.

(2) Water contaminated.

(3) Ion exchange capacity exhausted.

(4) Gas contaminated.

(5) Bleeding column.

(6) Leak in gas system.

Troubleshooting procedure:

(1) Attenuator at short, zero recorder.

(2) Change water.

(3) Change ion exchange bed.

(4) Check background with the cell disconnected from furnace, change gas.

(5) Replace column with glass jumper.

(6) Test system for leaks.

(b) Low sensitivity.

Symptom: noticeable loss from previous response.

Probable cause:

(1) Leak in flow system.

(2) Contaminated pyrolysis tube.

(3) Contaminated Teflon transfer line.

Troubleshooting procedure:

(1) Test system for leaks.

(2) Replace or re-cure pyrolysis tube.

(3) Replace Teflon transfer line.

(c) Noisy baseline.

Symptom: baseline noisy, 3 percent or more at x2 on attenuator.

Probable cause:

(1) Recorder gain too high.

(2) Bridge not properly grounded.

(3) Ion exchange capacity exhausted.

(4) Bridge defective.

(5) Dirty cell.

(6) Improperly cured column.

Troubleshooting procedure:

(1) Set bridge attenuator to x2. Adjust recorder gain.

(2) Connect a jumper from bridge white terminal to recorder ground.

(3) Change ion exchange bed.

(4) Substitute bridge.

(5) Clean cell with 10 percent solution of HF, rinse with distilled water.

(6) Recondition column.

(d) Loss of gas flow.

Symptom: bubbles not present in cell.

Probable cause:

(1) Gas tank empty.

(2) Broken pyrolysis tube.

(3) Broken column.

(4) Valve blocked.

(5) Broken flow control.

Troubleshooting procedure:

(1) Check tank pressure.

(2) Remove and inspect pyrolysis tube.

(3) Remove and inspect column.

(4) Check flow through valve and clean, if necessary.

(5) Check output from flow control.

(e) Loss of furnace heat.

Symptom: pyrometer does not read 820°C or the set temperature.

Probable cause:

(1) Heat control off.

(2) Thermocouple open.

(3) Furnace heater open.

(4) Heat control defective.

Troubleshooting procedure:

(1) Push heat control knob to turn heat on.

(2) Visually check furnace. Insure it is red on inside, check thermocouple with ohm meter.

(3) Check resistance of furnace heater with an ohm meter.

[1] (4) Check variable 1-110 VAC output of heater control with volt meter.

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[1] Caution: use only a volt-ohmeter. Do not use a vacuum tube volt meter.

(f) Loss of inlet block heat.

Probable cause:

(1) Heat control off.

(2) Thermocouple open.

(3) Block heater open.

(4) Heat control defective.

Troubleshooting procedure:

(1) Push heat control knob to turn heat on.

(2) Check thermocouple with an ohm meter.

(3) Check resistance of block heater with an ohm meter.

[1] (4) Check variable 0-110 VAC output of heater control with volt meter.

6M TROUBLESHOOTING THE FLAME PHOTOMETRIC DETECTOR (FPD)

(a) Noisy baseline [2]

Probable cause:

(1) Detector temperature too high.

(2) 750 volt power supply noisy.

(3) Noisy electrometer.

(4) Damaged photomultiplier tube.

(5) GC column bleeding.

Troubleshooting procedure:

(1) Lower temperature to 160-170°C.

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[1] See footnote on page 21.

[2] In any case of noisy baseline, make certain the recorder gain is properly adjusted and the slidwire is clean.

(2) Disconnect cable from end of PM tube and observe baseline. If noise continues, 750 volt power supply may be the cause. Repair or replace.

(3) Continuing noise if cable from back of electrometer is disconnected indicates bad electrometer.

(4) Continuing noise with extinguished flame indicates damaged PM tube if electrometer and power supply have checked good. Replace tube.

(5) Recondition or replace column.

(b) Low sensitivity

Probable cause:

(1) Dirty filter.

(2) Dirty window.

(3) Damaged photomultiplier; low sensitivity will probably be accompanied by excessive noise.

(4) Improper polarizing voltage.

(5) Light leaks.

(6) Improper flow rates.

(7) Loose cables.

Troubleshooting procedure:

(1) Remove filter and clean with lens tissue. Be sure to turn off polarizing voltage before removing PM tube.

(2) Remove PM tube and filter and look into back of detector. Replace window if coated with gray deposit.

(3) Replace PM tube.

(4) Perform voltage/injection profile.

(5) A shift in baseline will occur by shading the detector burner housing.

(6) Insure all flow rates are correctly set.

(7) Check that all cables are tight.

6N EPILOG

The reader is cautioned against immediately assuming that the source of an operating problem is an instrumental malfunction when in fact it may be something else entirely. For example, if the operator makes a series of injections of a relatively "dirty" extract for electron capture detection, such as the 15 percent ethyl ether-petroleum ether cleanup fraction of fat, certain symptoms may appear on the chromatogram which could suggest electronic problems. Peak height response may be greatly depressed, and significant peak tailing may occur. However, these manifestations are simply electronic symptoms and not causes. The cause of the problem in this case would be overloading and contamination of the GC column (see Figure 4-J in Section 4). Because of the visible similarities on the chromatograms of electronic vs. other problem sources, the operator should not proceed posthaste to disassemble the instrument without first checking out the possibility of other problem sources. In general, if the instrument has been performing satisfactorily up to the time of starting chromatography on a new series of samples, it would seem probable that the problem may reside in the sample extract rather than in the gas chromatograph.

Section 7

OTHER CHROMATOGRAPHIC DETERMINATIVE TECHNIQUES

HIGH PERFORMANCE LIQUID COLUMN CHROMATOGRAPHY (HPLC)

7A INTRODUCTION TO HPLC

High performance liquid chromatography is becoming increasingly important as a powerful technique for the separation and analysis of pesticide residues. HPLC is a very gentle technique that commonly operates at ambient temperature. It has advantages over gas chromatography where compounds are not naturally volatile and derivatization is difficult or unsatisfactory, and for polar or thermally labile compounds. Pesticides that may not gas chromatograph well unless derivatized (e.g., phenoxy acid herbicides, which require methylation prior to GC) often give excellent liquid chromatograms (1). The most widely used detector in LC, the ultraviolet (UV) photometer, is nondestructive to the sample, making fraction collection a routine matter. The separated compounds emerge from the chromatograph dissolved in solvent that can easily be collected and the solvent removed to recover the compound. A greater variety of separations of more complexity can be achieved by LC because of the active role played by the mobile phase as contrasted to carrier gas in GC. HPLC has been used for cleanup of pesticide extracts prior to GC determinations (2-4) as well as for the final determination itself.

Disadvantages of LC are that detector sensitivity is not comparable to that obtainable with GC detectors, especially electron capture, and a wide range of element selective detectors is not yet available. In general, present commercial LC detectors have sensitivities in the 10^{-5} to 10^{-9} g range. In one comparative study (1), detection limits by electron capture GC were 100-1000 times better for DDT and 2,4,-D than with an LC photometric detector, 5 ng of each pesticide being detected with the latter at 210 and 278 nm, respectively. However, the poorer sensitivity could be overcome by injection of large sample volumes (e.g., 50-100 µl) without loss of linearity or peak symmetry. The ability to introduce large volumes in LC can sometimes make sensitivity between the two methods comparable. Derivatization methods can increase the sensitivity of detection, e.g., by formation of UV-absorbing or fluorescent derivatives, but only at the cost of more complicated sample preparation. HPLC is still relatively new, and as improved equipment is developed and analysts obtain a better knowledge of HPLC, this technique will be taking its place beside GC and TLC as an important tool for pesticide residue determination.

Two terms that have evolved from the early traditions of liquid chromatography should be defined. In "normal phase" LC, the stationary phase (an adsorbent or partition medium) is more polar than the mobile phase, and the least polar sample components will elute first. In "reversed (or reverse) phase" LC, the stationary phase is less polar than the mobile phase, and the most polar sample components will elute first.

7B THEORY AND PRINCIPLES OF HPLC

The principles and theory governing GC and LC are very similar, but the presence of a moving liquid instead of a gas gives far different separation characteristics to LC. The choice of the carrier gas in GC is primarily dictated by the type of detector used and has little influence on the separation achieved in a given column. In LC, the composition of the mobile phase is of prime importance in the thermodynamic distribution process. The other significant differences between GC and LC are that, in the latter, solute diffusion in the mobile phase is extremely low and temperature effects are of only secondary importance. Low diffusion in the mobile phase (a factor of 10^4 less than in GC) is the key reason why HPLC is possible to perform. Instead of plate height becoming increasingly larger as the carrier liquid flow increases as in GC, it becomes asymptotic to a limiting value. In practical terms, this means that the mobile liquid phase velocity can be increased without the same great loss in efficiency (increase in plate height) and loss of resolution that occur in GC. Temperature changes can differentially alter the relative retention of two similarly retained solutes by the effect on solubility, mobile phase viscosity, mass transfer effects, etc., but these are only indirect effects, and most LC separations are carried out at ambient temperature.

The concepts of retention time and resolution are the same in LC as in GC. Good resolution requires that peaks be narrow and the distance between peak maxima be great enough to allow the trace to return as nearly as possible to the baseline. Peak width is a function of the column efficiency (number of theoretical plates), while peak separation is a measure of column selectivity (the ability of the packing to differentiate between two solutes).

The basic equations for HPLC are the following:

$$\text{Retention: } k' = \frac{V_1 - V_o}{V_o}$$

where V_1 is the retention volume for the peak of interest from the point of injection, and V_o is the retention volume of a non-retained peak measured at the peak apex. Times or distances measured along the recorder chart can be used more conveniently than volumes if the flow rate is constant.

Selectivity for compounds 1 and 2:
$$= \frac{k'_2}{k'_1} = \frac{V_2 - V_o}{V_1 - V_o}$$

No. of Plates:
$$N = 16\left(\frac{V_1}{W_1}\right)^2$$

where W_1 is the width of the peak for component 1 (see Figure 4-C) in terms of V.

Resolution:
$$R = \frac{1}{4}\underbrace{\left(\frac{\alpha - 1}{\alpha}\right)}_{\text{selectivity}}\underbrace{\left(\sqrt{N}\right)}_{\text{efficiency}}\underbrace{\left(\frac{k'}{1 + k'}\right)}_{\text{capacity}}$$

The k' term or capacity factor measures retention in column volumes; it is affected by the strength (e.g. polarity) of the solvent and strength (e.g. retentivity) of the column packing. The optimum value of k' is ca. 2-6. α is the separation or selectivity term, which is affected by the chemistry of the entire system, including the functionality of the sample components. Values of 1.1-2 are typical in HPLC.

N is a measure of band broadening; typically, a highly efficient small-particle, porous 25 cm silica gel column will show ca. 15,000 plates for various compounds. The resolution equation combines terms associated with selectivity, efficiency, and capacity.

If the resolution of two components with $k' \approx 2$ or less is unsatisfactory, there are three different ways to try to improve the separation. The solvent can be changed (solvent strength decreased) to give k' values between 2 and 6, the column can be changed to increase N and give narrower peak widths, or the solvent can be changed to give increased selectivity (α). As a specific example, an inferior reversed phase separation on a 37-75 μm bonded phase C_{18} column with methanol/water solvent might be improved by changing to a lower percentage methanol (increase k'), changing to a 10 μm column (increase N), or changing to acetonitrile/water solvent (increase α).

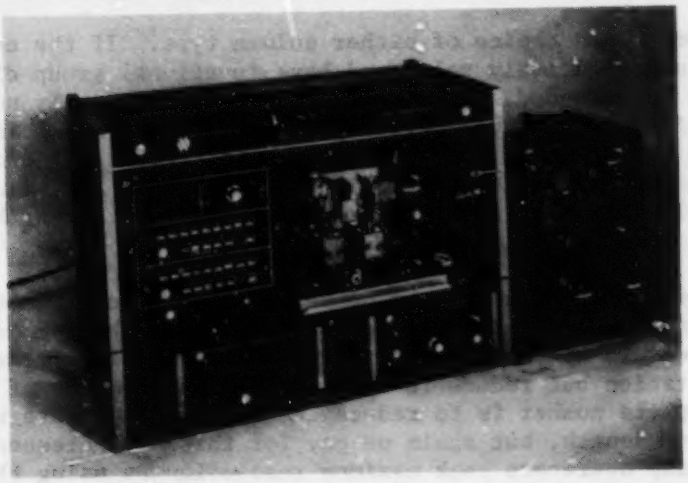
In general, the best order for developing a separation is the following: try to dissolve the compound(s) of interest in a series of solvents ranging from hexane to water. If the only solubility is in the hexane end of the series, choose a silica gel column; if the only solubility is in the water end, use a C_{18} bonded column. If there is intermediate solubility, one has a choice of either column type. If the compounds to be separated are relatively polar and have functional group differences, silica gel is recommended. If the compounds are relatively non-polar and differ mainly in the hydrocarbon skeleton, a C_{18} column is recommended. Use the best available column to increase N, choose a solvent mixture and vary its proportions to alter k', and, finally, change the solvent composition while keeping the same strength to increase α.

Doubling column length doubles the number of theoretical plates, but the separation time will also double if flow rate is kept constant. Increasing pressure with a constant column length will increase the speed of separation but reduce resolution. A simple means of increasing the plate number is to reduce the solvent flow rate with a constant column length, but again we pay for this by increased separation time. Many operators seek maximum resolution by using the longest column and highest flow rate feasible at the pressure limit of the available instrument. Decreasing the eluting strength (e.g., polarity) of the solvent will usually increase resolution but will also increase the analysis time. Column efficiency is only marginally affected by column diameter: there is a small increase with increasing diameter, but diameter is principally important to sample loading capacity (sample size is proportional to the grams of active stationary phase available in the column). Doubling column diameter will approximately increase capacity by four, but four times as much solvent must flow through the column in a given time to maintain efficiency and velocity. A guide to selecting the best experimental conditions for high resolution in HPLC with large- and small-particle columns has been published (5).

7C HPLC INSTRUMENTS

The basic elements of a complete, automated HPLC instrument include a solvent reservoir and gradient forming device, high pressure pump, injection system, column, detector, and recorder (Figure 7-A). The instrument components must be joined by tubing that is as short and as narrow in bore as possible with low dead-volume fittings and valves so as to minimize extra-column peak spreading. The gradient device mixes various solvents to produce a continuous or stepwise change in chemical composition (e.g., polarity or pH) of the mobile phase during the elution. Gradient elution is analogous to temperature programming in GC since both are used to speed and optimize complex separations. In adsorption and partition LC, the gradient usually involves an increase in solvent polarity; in reversed phase partition LC, solvent polarity is progressively decreased.

Figure 7-A High Performance Liquid Chromatograph Including Two Model 6000 Pumps, A Model 660 Solvent Programmer, and Model 440 UV Absorbance and R401 Differential Refractometer Detectors. Waters Associates.



The pumping system must provide the pressure required to achieve the correct flow rate through the column. Although most instruments permit pressures up to at least 5000 psi, the vast majority of analytical separations can be done at pressures ranging from a few hundred psi to about 1200 psi. HPLC pumps fall into two categories, namely, continuous displacement (e.g., gas displacement, gas amplifier, and syringe types) and intermittent displacement (e.g., peristaltic, diaphragm, and reciprocating piston). Most users and manufacturers today are emphasizing variations on the reciprocating pump, while other pump designs are fading into the background. The newest models feature highly precise flow, no significant pulsation of the final flow to the detector, and automatic correction to provide accurate flow under different operating conditions. The increasing use of microprocessors, which is a definite trend in HPLC equipment, allows improved operation of pumps and additional options such as low pressure gradient systems that use a proportioning valve in front of a single pump. Micro-processors also allow increasing automation of the entire LC system, including sample injection and data handling.

Injection is carried out in one of three ways in different commercial LC instruments. Stop-flow injection involves shutting off the flow of solvent in the column (either by stopping the pump or by using a shutoff valve), removing a cap from the head of the column, and injecting the sample directly on top of the column. Because diffusion in the liquid mobile phase is negligible compared to gaseous diffusion in GC, the cap can be resealed and chromatography can be resumed without significant loss of efficiency. Two different injector systems allow sample introduction from a syringe without stopping solvent flow. With a septum injector, the sample is injected directly into the flowing solvent with a technique similar to that used in GC. These injectors are most suitable for work at lower pressures. Septum deterioration from solvent attack and coring of septa during injection against high pressure are common problems with this type of system. With the septumless injector, the sample is introduced at atmospheric pressure into a loading loop that is being bypassed by the pump-to-column stream; by switching to the "inject position", the loop becomes part of the main solvent stream and the sample is immediately swept onto the column. The third type of injection system is a rotary injection valve in which an external loop is completely filled with sample, and the loop is then inserted into the flowing stream. It differs from the septumless syringe injector because the precise loop volume determines the amount of sample introduced rather than a syringe. A number of automatic devices for sequential introduction of multiple samples is also commercially available (see, for example, reference 6).

HPLC modules are connected together almost universally using 316 stainless steel. Although very hard and durable, these fittings can be damaged by too much tightening or many openings and closings of the liquid seal. With a new ferrule and nut, a leak-free seal is readily achieved because of the clean, polished, and level metal surfaces. After use, more torque is required for sealing, and ferrules and fittings become distorted. When a fitting is replaced, the ferrule connected to the tubing should also be changed. The ferrule is best removed by (a) scoring the 1/16 inch tube behind the ferrule with a sharp triangular file; (b) gripping each side of the scored tubing, 2 mm from the score, with pinch-nose pliers; and (c) bending the pliers toward and away from the score, through small arcs, until the tubing breaks at the score. This procedure assures the capillary bore of the tubing is still completely open. Any burrs should be removed with a file from the outer tubing edges before placing the new ferrule and nut on the tubing.

7D COLUMNS FOR HPLC

Column efficiency is increased by using columns that are densely packed with uniform, small particles. Particles with an average size down to 30-40 µm can be successfully dry packed in the laboratory while microparticulates (5-10 µm) must be slurry packed. Because of the difficulty of this operation, commercial, pre-packed columns are usually used.

Pellicular packings have a thin layer or shell of stationary phase bonded to a solid glass core. The active layer can be silica, alumina, an ion exchange resin, or silica gel to which a "liquid" phase has been bonded (bonded phase partition packings). The thin layer of stationary phase provides good mass transfer (efficiency) with a particle diameter that allows dry packing and low inlet pressure operation. A disadvantage of the thin active surface is reduced sample capacity. Totally porous microparticulates have very high efficiency because of their small average diameter (5-10 µm) and also have higher capacity than pellicular packings. However, they require high inlet pressure for acceptable flow rates.

For liquid-solid adsorption chromatography, microparticulate silica gel of spherical shape is recommended. Alumina or other packings are used occasionally. Bonded phase packings are used for normal and reversed phase liquid-liquid partition separations. Reversed phase chromatography on a monomeric or polymeric phase consisting of C_{18} linear hydrocarbon covalently bonded to silanol groups of silica gel particles is by far the most widely used LC mode. Fully aqueous or aqueous-organic solvent mixtures are used as the mobile phase. Other reversed phase bonded packings have phenyl and cyclohexyl groups, while normal phase bonded packings contain polar functionalities (e.g., nitrile, amine). Anions are separated on silica-based or resinous anion exchange columns and cations are separated on cation exchange columns. Instead of ion exchange, ionic compounds may be better separated by ion pair chromatography. In this technique, ionized samples interact with an oppositely charged counter ion in the mobile phase to produce a neutral ion pair, which is selectively retained by a C_{18} reversed phase column. Gel permeation or exclusion chromatography is used mostly for the cleanup of pesticide extracts. High molecular weight lipid impurities are eluted as a group before the smaller pesticide molecules.

Reversed phase HPLC on bonded C_{18} packings owes its great popularity to its ability to separate nonionic, ionic, and ionizable substances in partition, ion suppression, or ion pairing (7) modes; the stability of the bonded phase columns (if properly used); and the simple, inexpensive solvent systems utilized such as methanol/water or acetonitrile/water mixtures. Disadvantages of reversed phase LC on bonded packing include unreacted silanol (Si-OH) groups that can lead to peak tailing of polar, and particularly basic, substances. This is overcome by adding a competing base to the mobile phase or employing ion pair chromatography. Another limitation is the lack of clear understanding of the retention of polar and nonpolar solutes on these columes. A detailed discussion of HPLC columns and column technology has been published (8).

Microparticulates of 5 µm and 10 µm average particle diameter are the currently preferred packing for HPLC. The 10 µm particles provide adequate resolution for many separations, while 5 µm particles are recommended for the most demanding separations. In general, 5 µm particles give 2-3 times more theoretical plates than 10 µm particles packed in the same length column, but backpressure is 3-4 times higher. Therefore, lower flow rates are recommended for smaller particles. For example, 2 ml/minute is a typical flow rate for a 4 mm id column packed with 10 µm particles, while 0.5-1 ml/minute would be used for the same column packed with 5 µm particles. Pellicular packings are mostly useful for guard columns to protect the analytical column from contamination (see Subsection 7G below). For this purpose, pellicular packings can be easily dry packed.

Prepacked columns are available with inner diameters ranging from 2 mm to 8 mm. For most analytical and small-scale preparative work, a 4 mm id column is recommended. For 5 µm particle diameter packings, 15 cm, 25 cm, and 30 cm column lengths are available. The 15 cm column is recommended for simpler separations requiring only a few thousand theoretical plates. It uses less solvent, gives twice the separation speed, gives slightly higher pressure drop, has half the capacity, but gives about the same number of plates as a 30 cm column packed with 10 µm particles. Compared to 30 cm, 5 µm particle columns, 15 cm, 5 µm columns give half the number of plates, pressure drop, separation time, sample capacity, and solvent usage. A good 15 cm, 5 µm particle column should produce ca 9,000 plates, a 25 cm column 15,000 plates, and a 30 cm column 18,000 plates.

An important consideration for trace analysis is that much larger sample volumes can be injected for HPLC compared to GC. Injection volumes of 50-100 µl are not uncommon for an LC column. This may eliminate the sample concentration step often required prior to GC, and can help to offset the lower sensitivity of LC detectors. Wider bore columns are better for the injection of larger samples since the allowable sample volume is proportional to the square of the tube diameter. In general, the sample should be injected in the mobile phase or in a solvent that is significantly weaker than the mobile phase so that preconcentration of the solute occurs at the top of the column. For gradient elution, injection of larger volumes of a dilute solution in the initial (weaker) solvent can be made, or a very low volume of sample dissolved in the final solvent of the gradient.

7E MOBILE PHASES (SOLVENTS) FOR HPLC (see also Subsection 3I)

Solvents chosen for adsorption HPLC should have a low viscosity (for high efficiency and low backpressure), low boiling point (to facilitate sample recovery), adequate purity, low toxicity and odor, reasonable cost, and detector compatibility (low UV cutoff for the popular UV absorption detector). A widely versatile set of solvents with a range of chromatographic properties is hexane, methylene chloride, diethyl ether, acetonitrile and methanol (most polar -- strongest eluant for normal phase adsorption chromatography). These solvents are usually used in mixtures with a solvent composition and strength that optimize capacity and selectivity (Section 7B). Solvent mixtures for adsorption HPLC of polar samples often contain at least a small concentration (0.01-1%) of a polar modifier (e.g., water, alcohol, acetonitrile) (9). Dissolved oxygen in solvents can have a variety of undesirable effects on UV and fluorescence detectors. Helium sparging of solvents is an effective way to remove oxygen and eliminate artifacts it can cause (10).

Many of the same considerations apply to the selection of solvents for the most important HPLC mode, reversed phase chromatography on chemically bonded packings. Because the phases are reversed, water, the most polar solvent, is the weakest eluant, while neat methanol and acetonitrile are the strongest eluants used in most applications. The most polar solutes are the least retained on the column. Eluants of intermediate polarity are usually obtained by mixing methanol or acetonitrile with water or an aqueous acid, base, or buffer solution (11). Ternary solvent mixtures of varying composition, e.g., water, methanol, and acetonitrile or tetrahydrofuran (12), allow control of selectivity of solutes with different functional groups and provide the usual discrimination of the nonpolar portions of the molecules typical of reversed phase LC. In general, silica gel bonded reversed phase packings are stable only with solvents in the pH range of 2-7.5. At low pH, attack of the Si-C bond is possible, whereas at high pH, the silica matrix may be attacked, particularly in salt solutions. In both adsorption and reversed phase partition HPLC, gradient elution can optimize both resolution and speed for complex samples with components that cover a broad range of polarity [but resolution of any given compound pair is actually reduced compared to isocratic (constant solvent composition) elution (13)].

Solvents for separation of ionic compounds (e.g., pesticides with acidic or basic groups) by ion exchange chromatography include aqueous acids, bases, or buffers that allow the solutes to possess full or partial electronic charge and to be more or less attracted to the ionic groups of the stationary phase. As an alternative, the pH of the mobile phase can be adjusted with acids, bases, or buffers to reduce or eliminate the degree of ionization of acidic or basic groups and allow separations by a partition mechanism on a C_{18} bonded phase column. This method is termed ion suppression. A third mode for the separation of electrolytes is ion pairing. The mobile phase is at a pH where the solute is charged, and it also contains a pairing agent that conjugates with the solute to form a hydrophobic, uncharged species that is selectively retained by a C_{18} bonded phase. Typical pairing agents are a quaternary amine for weak acids and an alkyl sulfate or sulfonate for weak bases. The chain length of the counter ion controls the hydrophobicity of the final ion pair and, therefore, the extent of retention by the column.

Solvent selection for a particular separation is aided by two fundamental parameters, namely solvent strength (for control of k') and solvent chemistry (for control of α). Solvents have been tabulated according to their strength (polarity index) and chemistry (solvent group) for easy comparison (14). A rational approach to the selection of mobile phases for all forms of HPLC has been presented (15).

When changing from one solvent to another, time must be allowed for the column to become fully equilibrated with the new solvent. For bonded phases this will require only about 5 column volumes, but for adsorbents and some ion exchange resins, the equilibration volume can be large (ca 30-50 column volumes).

7F DETECTORS FOR HPLC

The most common detector for HPLC is the ultraviolet (UV) absorbance detector. The original UV detector used a low pressure mercury lamp with a filter and emitted light of very high intensity predominantly at a wavelength of 254 nm. Many aromatic compounds absorb strongly at or near this wavelength and so can be detected with good sensitivity (ca 10^{-10} g/ml) with this detector. It is advantageous, however, to have a variable wavelength spectrophotometer-type detector so that the analyst is able to work at the wavelength of maximum sensitivity for each compound, increase selectivity of detection of the analyte over interferences, and use absorbance ratios at several wavelengths to improve identification of peaks. Sources for variable wavelength detectors include a phosphor-coated low pressure mercury arc, a medium- or high-pressure mercury lamp, or a xenon-arc lamp, each combined with interference filters or a monochromator for isolating the desired wavelength. Since the intensity output of the variable wavelength detector is lower than the mercury arc, sensitivity of detection is somewhat lower for those compounds with a strong absorptivity at 254 nm.

Refractive index detectors are either the optical deflection or the prism (Fresnel) type. These are universal, relatively insensitive (10^{-6}-10^{-7} g/ml) detectors that require close temperature control (± 0.5°C) and cannot be used with solvent programming. They have had little or no use in pesticide residue analysis.

Fluorescence detectors are highly sensitive (10^{-10} g/ml) and selective because of the choice of excitation and emission wavelengths. An especially promising approach for trace analysis is the use of high intensity laser sources, with which 750 fg of aflatoxins have been detected (16). Derivatization (pre- or postcolumn) of the pesticide of interest is often required for increased selectivity or sensitivity of detection.

The first electrochemical detector for HPLC utilized polarography, but recent models have employed amperometry, coulometry, or conductivity. Reference (17) reviews the theory, applications, advantages, and disadvantages of these detector types. The amperometric detector as developed by Kissinger and co-workers seems especially promising for pesticide analysis, having picomole sensitivity and selective response (18). Seven halogenated aniline metabolites of carbamate, urea, and anilide pesticides have been separated without derivatization on a C_{18} bonded phase column and detected at levels of 0.23-0.38 ng (to give a response that is twice the noise level) using a commercially available electrochemical detector (model LC-2A, Bioanalytical Systems, Inc., West Lafayette, IN) operated with a + 1.1 volt oxidation potential (19). This detector cannot be used with flow or solvent programming, and waiting periods of 10 minutes to several hours are required for any changes in conditions (flow rate, applied voltage, different solvents) or for initial start-up each day. The sensitivity of the detector varies drastically with applied voltage, and voltage can be varied to obtain selectivity for analytes over interferences. Both increased flow rate and an increase in the volume of sample injected decrease detection sensitivity. Detector response was linear from the detection limit up to ca 50 ng injected.

The traveling wire flame ionization detector gives promise of universality and sensitivity like its GC counterpart, but it is at present mechanically complex and cumbersome. The electron capture detector has been adapted for HPLC using mobile phases that do not give responses. The column effluent is vaporized directly into the detector, in an atomized form, by means of a heated transfer tube located in an oven. Nitrogen is used as the purge gas to sweep the vapors out of the detector (20). LC-EC systems are commercially available with sensitivities listed as 10^{-10} g/ml for common chlorinated pesticides. The FPD (22) is similar to the detector used in GC but utilizes a special burner assembly to handle the total liquid effluent of the column, which is nebulized and directed into the cool hydrogen-nitrogen flame. Emission is measured by a simple bandpass photomultiplier system using the usual S- and P- wavelengths. Sensitivity is limited by the quenching effect of the organic solvents used as the mobile phase. Practical analyses of pesticide residues have not been reported with any of these detectors.

7G PRACTICAL ASPECTS OF SUCCESSFUL HPLC OPERATION

All new columns should be tested with a standard mixture at standard chromatographic conditions to compare with the manufacturer's guarantee or previously used columns, and as a reference point for monitoring column changes with use. New columns must be fully equilibrated with the solvent, and the column connections must have zero dead volume if constant retention times and high efficiency are to be achieved. The test mixture should contain pure compounds, one of which is nonretained plus at least two others that have $k' < 10$ and are well resolved so that as the column slowly degrades they will not overlap. The test mixture can contain pesticides to be analyzed in real samples with the same solvent system that is to be employed, or it can be a mixture specified by the column manufacturer so the performance data supplied with the (pre-packed) column can be verified. The concentration levels should be comparable to those to be used in the actual analysis.

Parameters monitored include absolute (k') and relative (α) retention, plate number (N), asymmetry (tailing), void volume, and pressure drop. Small differences in α and k' usually reflect normal differences in solvent composition, but large decreases in these parameters or an increase in asymmetry are indications of column degradation or deactivation. Both channeling and compression of the packing can also increase. If the void volume decreases, channeling may be occurring or the packing pores may contain gas bubbles or immiscible liquid. Changes in pressure drop indicate channeling, plugging, or leaking. Buildup of impurities from the solvent or samples will eventually cause loss of column efficiency, which can usually be restored by regenerating the column with a series of solvents of increasing eluting strength (adjacent solvents must be miscible). The solvent sequence is then reversed, and each solvent is followed by a weaker one. A possible solvent sequence for regenerating adsorption (normal phase) columns is methylene chloride-methanol-water-methanol-dry methylene chloride-dry hexane (20 column volumes each). Acetonitrile containing 1% DMSO or DMF (20 column volumes) is an effective solvent for regenerating reversed phase bonded packings. A column volume or dead volume is 50-60 ml for a typical 4 mm id x 25 cm HPLC column. The pump and connecting tubing are prewashed with each new solvent that is put through the column, a flow rate of ca 2 ml/minute is used, and the detector is left connected, if possible, to also clean it. Regeneration of HPLC columns has been described in detail (23).

The inevitable, permanent column degradation that occurs with prolonged use can be retarded if proper precautions are taken for sample cleanup, solvent preparation, periodic column regeneration, and storage. The manufacturer's literature should always be carefully studied and recommendations should be faithfully followed. Prevention of plugging is probably the one most important precaution that must be exercised to prolong column permeability and efficiency. Removal of particles from solvents is discussed along with other aspects of solvent purity in Section 3I. Sample extracts or solutions should also be free of insoluble particles (24) and should be filtered, if necessary, with a hypodermic syringe fitted with a Swinny-type filter (0.5-1 μm). Irreversibly sorbed compounds can irreparably damage the column and, if present, can be removed on a short (5-10 cm) guard column located between the injector and the analytical column. In order not to sacrifice separation efficiency, the guard column should be of the same diameter and packing as the main column. Less expensive, easily dry-packed guard columns can be prepared from ≈40 μm pellicular sorbents, but some efficiency may be lost if the analytical column contains microparticulates. In addition to the guard column, a silica precolumn (25) should be placed between the pump and the injector to presaturate the mobile phase with silica gel and retard the dissolving of HPLC columns. Solubility of silica gel leads to sunken beds with skewed surfaces, resulting in distorted peak shapes or increased backpressure. The extent of dissolving is a function of the type of column packing and the exact nature (pH, concentration) of the mobile phase. When placed before the injector, voids do not contribute to band broadening and loss of efficiency, so that large particle silica gel is perfectly adequate.

Columns damaged by plugging, bed compression, or irreversibly sorbed material can sometimes be returned to original efficiency by removing the column inlet fitting and frit and replacing the discolored packing and deposited material with fresh packing. The same packing material should be added by the appropriate dry or slurry packing procedure, or, alternatively, a methanol slurry of the packing can be added dropwise and allowed to settle into place. The end frit is cleaned before replacement by immersion in an ultrasonic bath for a few minutes.

A properly packed and cared-for column should be stable for 3-6 months or more with continual use. A poorly packed column can settle with use, creating a void at the top that leads to broad peaks with poor symmetry. Purchase of a good commercial column or properly performed slurry packing followed by column compaction via pressure pulsing (26) should virtually eliminate this problem. HPLC columns should never be bumped, dropped, jarred, bent, tapped, or vibrated. All connection fittings must be clean (use an ultrasonic bath). Fittings are never over-tightened or they will become distorted and eventually leak. Columns should be stored tightly capped in a compatible solvent. Silica-based bonded reversed phase packings are used between pH 2 and 7.5 and are never stored in aqueous solution but are flushed and stored in methanol or acetonitrile. If the column has been used with a buffer, it is flushed with water and then with the organic solvent. Aqueous solutions can be left flowing slowly overnight (5 ml/hour) for use the next day, but the column should not remain static in aqueous solution.

Some common problems in adsorption LC and possible means for their correction (9) follow. Baseline drift can be caused by strongly adsorbed peaks eluting from an earlier run. Such drift is remedied by pumping through the column at the end of each run several column volumes of strong solvent (isocratic elution) or solvent of higher final strength (gradient elution). Baseline drift can also be caused by incomplete system equilibration in switching from one solvent to another. This problem is most acute with the RI detector. Spurious peaks can be caused by bubbles in the detector or impurities in water or other solvents. Bubble formation is avoided by prior solvent degassing or installation of an in-line backpressure valve generating 50 psi to keep all gases in solution. Peak tailing is more common in adsorption HPLC and is often caused by insufficient adsorbent deactivation. Use of a modifier in the solvent can correct this problem. Partial ionization of the sample can cause tailing that can be suppressed by changing the solvent pH or ionic strength. Injection of the sample in a solvent stronger than the eluant can also cause tailing; a solvent weaker than the eluant, or more preferably the eluant itself, should always be used, if possible, or the sample may be injected in a very low volume of a stronger solvent. In reversed phase systems, peaks can also be broadened or even split when the sample is injected into a mobile phase that is either more or less polar than the solvent in which the sample is dissolved (27). Drifting retention times can be caused by differences among solvent batches, changes in composition of a batch of solvent on standing, changes in temperature, or inconsistent adsorbent activity. Adsorbent activity is maintained constant by using clean samples, pure solvents with an adequate level of modifier, and frequent column regeneration. Poor reproducibility of retention times and peak areas in gradient elution may arise from inadequate column regeneration between runs or poor mixing in the mixing chamber. Other causes of nonreproducible retention times can be a nonconstant recorder drive or slipping chart paper; a nonconstant flow rate of mobile phase caused by nonreproducible pump delivery or a leak at any fitting throughout the system or in the injector or pump; or a contaminated or "coated" column that is irreversibly retaining a portion of the sample and thereby changing the partition coefficients of some of the components.

Dirty detector flow cells may be cleaned by using a 10 ml syringe to rinse the cell successfully with methylene chloride, methanol, and water. The cell is then filled with 50% nitric acid and allowed to stand for 30 minutes, followed by flushing with filtered, distilled water (28). If this operation does not reduce the background to an acceptable level, either other problems are involved (e.g., cell misalignment or an impure or inappropriate solvent) or the detector must be disassembled and cleaned further in accordance with the manufacturer's instructions.

Quantitation in HPLC with UV detection is best carried out using peak heights if solvent composition can be maintained precisely but flow control is poor. Peak areas are used when flow rate is stable but the composition of the mobile phase might vary (as would be common in adsorption chromatography where traces of water and polar contaminants are difficult to control) (29).

Readers using HPLC for analysis of pesticide residues are strongly urged to study the excellent discussion of many practical aspects of the field given in reference (9), from which much of the material in this subsection was taken.

7H HPLC DATA

HPLC data of 166 pesticides in the form of elution volume or capacity factor (k') have been tabulated (30). Information has been included on the column packing and dimensions, mobile phase composition, detector, and sample substrate. Data for 26 urea herbicides on silica gel 60 with hexane-methylene chloride-ethanol (20:79:1 v/v) were also reported (31).

7I APPLICATIONS OF HPLC TO PESTICIDE ANALYSIS

The material presented on HPLC has been expanded in the present revision of this manual because of the increasing importance of the technique in residue analysis. Even greater coverage is anticipated in future revisions as HPLC becomes more sensitive, practical, and reliable in the multiresidue analysis of field samples. For further information on HPLC, readers are referred to reviews describing LC detectors (32-34), column packings (8), and general principles and equipment (35,36), and to books covering theory, principles, and practice (37,38). A scheme has been published for isolating and troubleshooting instrument and column problems in HPLC utilizing a glass-bead column and two simple electronic checking devices (39).

A novel method with great promise for the simplified monitoring of residues in water samples down to ppt levels has been termed "trace enrichment". The procedure combines concentration, separation, detection, and quantitation of nonpolar to moderately polar impurities. The water to be analyzed is pumped through a C_{18} reversed phase column until a sufficient quantity of impurities has been deposited at the top of the column. (In a reversed phase system, water is the weakest possible eluant, so the organic compounds will be concentrated in a tight band at the head of the column.) A gradient elution from 100% water to 100% methanol or acetonitrile is then performed, during which the organic impurities are eluted sequentially in order of their polarity (most polar is first eluted) and detected with a UV absorption detector (40).

Table 7-1 contains some other recent applications of HPLC to pesticide analysis, selected to illustrate the range of pesticide types and samples that have been studied. Most analyses to date have been developed for one or a few specific residues in food or crop samples, and many analyses of formulations and technical material now involve HPLC as the determinative step. Review of applications of HPLC to pesticide analysis are cited in References (1, 41-44).

An investigation was carried out to assess the usefulness of reversed phase HPLC with UV detection and gradient elution for the determination of residues of pesticides included in the European Economic Community directive on fruits and vegetables. It was shown that most of the 42 compounds, comprising pesticides of many different chemical classes, could be separated and detected, but that sensitivity was not sufficient to detect some compounds at or near the EEC limit (45). The best application of HPLC was considered as an adjunct to established GC procedures for multiresidue screening. Applications of HPLC in the environmental analysis of water were reviewed (46). Preconcentration and cleanup of residues on small, disposable Sep-Pak cartridges (Waters) allowed the determination of 20 pesticides at 20 ppb levels in surface water.

Attempts have been made to combine HPLC and mass spectrometry for the direct analysis of column effluents. Approaches to this direct coupling have included (a) use of atmospheric pressure ionization; (b) enrichment of reversed phase effluents using a dimethyl silicone membrane interface; (c) chemical ionization using a small fraction of the carrier solvent as reagent gas; (d) transport of solute through differential vacuum locks on a wire or a metal or plastic ribbon; (e) reduction of solute to hydrocarbon and sebsequent FID and MS analysis; and (f) formation of a molecular beam by laser vaporization of solvent. At present, (c) and (d) are the most popular methods, but none is free from major disadvantages and none has yet been tested for routine pesticide analysis. The various methods for on-line and off-line coupling and some applications of HPLC-MS have been reviewed in detail (47-49).

THIN LAYER CHROMATOGRAPHY

7J INTRODUCTION TO TLC

The first multiresidue method available to the pesticide analyst for identification and estimation was based on paper chromatography (94-96). Paper chromatography has now been largely replaced by thin layer chromatography (TLC) since the latter will generally give faster and more efficient separations with better spot definition and greater sensitivity. The use of paper chromatography in pesticide residue analysis has been reviewed (96-99).

TLC is used mainly for confirmation of residues following initial screening and quantitation by GC. Confirmation by TLC, which is based on comparison of migration distances of the pesticide of interest with authentic standards run on the same layer, is covered in Section 10E of

TABLE 7-1 SELECTED SEPARATIONS AND DETERMINATIONS OF PESTICIDES BY HPLC

Compound determined	Sample matric	Column type	Solvent system	Detector	Cleanup	Recovery	Residue level	Reference
Abate	water	diphenylsilane reversed phase	water-acetonitrile gradient	UV, 254 nm	none	98-102%	5-150 μg/L	(90)
Azinphos-methyl oxon	foliage, soil	Corasil-uC_{18}, reversed phase	Acetonitrile-water gradient	UV or on-line colorimetric reactor	none	96%	1-10 ppm (soils)	(58)
Benomyl and TBZ	plant tissues	20-23 μm silica gel, adsorption	0.1% acetic acid in methanol	fluorescence	benomyl hydrolyzed to 2-AB	90-103%	ca 0.01-0.2 ppm	(66)
Carbamate insecticides	standards	μBondapack C_{18}, reversed phase	methanol-water gradient	fluorescence, after post-column labeling with o-phthalaldehyde	--	(3.5% C.V.)	ng levels	(74)
Carbamate insecticides and metabolites	vegetables	C_{18} reversed phase		fluorescence, after dynamic hydrolysis and labeling	partition	69-90%	0.01-1 ppm	(51)
Carbamate pesticides (Swep, Landrin, carbofuran, aminocarb, Banol, carbaryl, Zectran, methiocarb, propoxur, Mobam)	foods	5 μm silica gel, adsorption	5% isopropanol in isooctane	UV, 254 nm	partition and Florisil column	>70%	0.1-0.3 ppm	(63)
17 Carbamate and urea pesticides	standards only	Partasil-10 ODS reversed phase	different polarity solvents	UV, 254 nm	--	—	--	(53)

Carbamate insecticides (see OCl, OP, carbamate insecticides)
Carbamate pesticides (see Phenylurea, carbamate, thiocarbamate pesticides)

TABLE 7-1

Carbaryl	honey bees	Permaphase C_{18}, reversed phase	methanol-pH 7 phosphate buffer (4:1)	fluorescence	Florisil column	--	0.01 μg/bee	(93)
Carbaryl	potato, corn	silica gel 60	isooctane-isopropanol (96:4) or-dioxane (95:5)	UV, 254 nm or fluorescence after dansylation	partition and 5% water-deactivated Florisil column	78-90%	10-100 ppb	(79)
Carbofuran and metabolites	crops	silica gel 60	isooctane-isopropanol (97:3)	fluorescence after dansylation	partition and 2% water-deactivated Florisil column	50-65%	0.01 ppm	(83)
Carbofuran and 3-OH and 3-keto metabolites	crops	5 μm silica gel, adsorption	3-8% isopropanol in isooctane	UV, 254 or 280 nm	partition and Florisil column	68-110%	0.1 and 1 ppm	(52)
Chlortoluron	soil	10 μm silica gel, adsorption	15% isopropanol in hexane	UV, 240 nm	silica gel column	71-95%	0.25-2.5 ppm	(70)
Difenacoum and Warfarin	liver, plasma, urine	Corasil II pellicular, adsorption	isopropanol-$CHCl_3$-isooctane (1:2:397)	UV	GPC or partition	62-90%	0.025-5 ppm	(55)
Diflubenzuron	environmental samples	μC_{18}, reversed phase or μPorasil silica gel	methanol-water (75:25) or isooctane-isopropanol (93:7)	UV, 254 nm	partition and column adsorption chromatography	70-128%	0.01-0.05 ppm	(78)

DCPMU (see Methazole, DCMU, and DCPU)
DCPU (see Methazole, DCPMU, and DCPU)
Diquat (see Paraquat or diquat)

TABLE 7-1

Dioxins	PCP	Permaphase-ODS, reversed phase	14% H_2O in methanol	UV	extraction, ion exchange column	93-104%	0.4-41 ppm	(68)
Ethoxyquin	apple extracts	Spherisorb 10 ODS, reversed phase	water-methanol (20:80)	fluorescence	alumina column	98%	0.05 ppm	(73)
Ethylenethiourea	foods	Micropak SI-10 silica gel	isopropanol-methylene chloride (3:97)	UV, 254 nm, for the pentafluorobenzamide derivative	alkylation and alumina and silicic acid columns	>80%	0.1-5 ppm	(81)
5 Fungicides (benomyl, phenylphenol, biphenyl, thiabendazole, carbendazim)	fruits	adsorption, reversed phase, ion exchange	various, compatible with column	UV, 254 or 288 nm	steam distillation, partition, chemical reaction	64-102%	0.8-100 ppm	(54)
Hydroxy-s-triazines	plant material	silica gel 60	chloroform-methanol-water-87% H_3PO_4 (700:300:60:1)	UV, 240 nm	ion exchange or gel column	70-113%	0.05 ppm	(92)
Karbutilate and metabolites	water, soil, grass	μPorasil, adsorption	3-7% ethanol in ethylene dichloride	UV, 254 nm	Florisil column (for grass extracts)	80-103%	0.1-0.2 ppm	(60)
Methazole, DCPMU, and DCPU	plant and animal samples	Zorbax SIL, adsorption	0.05% methanol in dichloromethane or $CHCl_3$-petroleum ether-methanol (6:3:0.5)	UV, 254 nm	partition and silica gel column	75-105%	0.1-0.2 ppm	(71)
Methomyl and oxamyl	vegetable crops	μC_{18}, reversed phase	acetonitrile-phosphate buffer (11:89)	UV, 240 nm	partition	61-81%	2 ppm	(77)
Methyl parathion	runoff water	Partasil ODS, reversed phase	acetonitrile-water (50:50)	UV, 270 nm	XAD-2 for preconcentration	99%	2-3 ppm	(56)

TABLE 7-1

Naphthaleneacetic acid	oranges, tangerines, processed products	µCN and ETH bonded reversed phase	pH7 phosphate buffer and pH 4 citrate buffer	fluorescence	partition	64-99%	0.008-1 ppm	(87)
Naphthaleneacetic acid	apples	μC_{18} or µLiChrosorb NH_2	acetonitrile-water (20:80)	UV, 220 nm or fluorescence	acid-base partition	86-98%	0.01 ppm	(85)
4-Nitrophenol and other phenolic metabolites	urine	μC_{18}, reversed phase	methanol	UV	acid hydrolysis, steam distillation, automated	not given	1-2 ppm	(84)
OCl, OP, carbamate insecticides	grape extracts	C_{18}, reversed phase	water-acetonitrile (1:1) plus 10% phosphate buffer	UV, 221 nm	None	not given	0.002-0.2 ppm	(72)
Paraquat or diquat	urine	α- amino-propyltri-ethoxysilane bonded to 20 µm alumina	pH 2.45 phosphate buffer-methanol (11:14)	UV, 258 nm	none	ca 100%	650 µg/L	(67)
PCBs	commercial mixtures	µm silica gel, adsorption	dry hexane	UV	—	--	--	(64)
Various pesticide classes	standards	BioBeads SX-4, Merckogel OR-5000, Sephadex LH-20	ethanol, propanol, THF	UV	--	--	0.5-5 µg	(80)
Phenylurea, carbamate, thiocarbamate pesticides	surface water	silica gel 60	hexane-chloroform (4:1)	UV, 254 nm	extraction, ion exchange, and reversed phase columns	not given	10-20 ppb	(89)

OP insecticides (see OCl, OP, carbamate insectides)
Oxamyl (see Methomyl and oxamyl)

TABLE 7-1

Phenylurea herbicides	soil, river water	Spherisorb ODS, reversed phase	methanol-water-NH_3	UV	none	90-100%	0.5-1 ppm	(59)
Pirimiphos methyl and metabolites	plasma and urine	mixed, short-alkyl-chain bonded reversed phase	methanol-pH4 phosphate buffer	UV, variable wavelength	deproteinization	--	2 ppm	(86)
Pyrazon	water	C_{18}, reversed phase	methanol-acetic acid gradient	UV, 270 nm	none	>90%	200 µg/L	(88)
Rotenoids	plant extracts	C_{18}, reversed phase	acetonitrile-water (35:65) to 100% acetonitrile gradient	UV, 254 and 294 nm	partition and silica gel column	not given	not given	(75)
Rotenone and degradation products	animal chow and tissue	C_{18}, reversed phase	methanol-water (75:25)	UV, 295 nm	partition and silica gel column	51-92%	0.5 ppm	(82)
TH-6040 insect growth regulator	bovine manure	C_{18} reversed phase	acetonitrile-water (57:43)	UV, 254 nm	Florisil column	97%	0.5 and 2 ppm	(50)
Terbutryne	water	Permaphase-ETH, aliphatic ether, chemically bonded pellicular	20% methanol in water	UV	none	89-100%	0.001-0.1 ppm	(69)
Triazine, urea, uracil herbicides	standards	PE C_{18} Sil-X-11, reversed phase	2.5-25% methanol in water	UV, 200-300 nm	—	—	Sensitivity 0.05 ppm in soil and 0.001 ppm in water stated	(61)

Thiocarbamate pesticides (see Phenylureas, carbamate, thiocarbamate pesticides)
TBZ (see Benomyl and TBZ)

TABLE 7-1

Urea herbicides	soil, foods	Vydac ODS 10 μ, reversed phase	methanol-water (1:1)	UV, 254 nm	none	--	5-20 ng	(91)
Urea herbicides (linuron, chlorbromuron, chlroxuron, fenuron, etc.)	vegetables and wheat	5 μm silica gel, adsorption	5-20% isopropanol in isooctane	UV, 254 nm	partition and Florisil column	>80%	0.01-1 ppm	(65)
Warfarin	biological fluids	Micropak CH-10	methanol-0.5% acetic acid (1:1)	UV, 308 nm	freezing of aqueous phase	(2-5% C.V.)	0.1-4 μg/ml	(57)
Warfarin	plasma	10 μm silica gel	dioxane-water (2:3), pH 4.2	UV, 305 nm	none	82%	0.5 μg/ml	(76)
Warfarin and metabolites	blood, plasma, and liver microsomes	μC_{18}, reversed phase	1.5% acetic acid-acetonitrile (69:31)	UV, 313 nm	on-column concentration	≈100%	0.04-0.08 μg/μl	(62)

Urea herbicides (see Triazine, urea, uracil herbicides)

Urea pesticides (see Carbamate and urea pesticides)

Warfarin (see Difenacoum and Warfarin)

this Manual. In addition, TLC may be used as a screening procedure followed by confirmation and quantitation using GC, or the quantitation can be carried out by TLC if a gas chromatograph is not available or if the pesticide of interest is unstable during GC. Extraction, cleanup, and concentration steps normally precede TLC determination. Often more stringent cleanup is required for TLC than for GC if streaked zones are to be avoided. For example, the 15% diethyl ether fraction from the Florisil column cleanup of a fat sample contains a large amount of lipids. Although adequate for GC, further cleanup prior to TLC is required (EPA PAM, Section 12,B,V). TLC has also been occasionally used for cleanup of extracts prior to determination by GC (100).

Major advantages of TLC are simplicity, rapidity, and low cost. Sensitivity ranges from about 5-500 ng for most pesticide detection methods. Rapid semi-quantitative estimation can be achieved by visual comparison of sample and standard spot sizes and/or intensities, and more accurate quantitation can be carried out by in situ scanning of spots with a spectrodensitometer.

This section will briefly survey important aspects of TLC for screening and quantitation of pesticide residues. General techniques of TLC were described in detail in an extensive treatise (101), while specific procedures for pesticide TLC were covered in several papers (97, 102). Applications of TLC to pesticide analysis have been reviewed (103, 104).

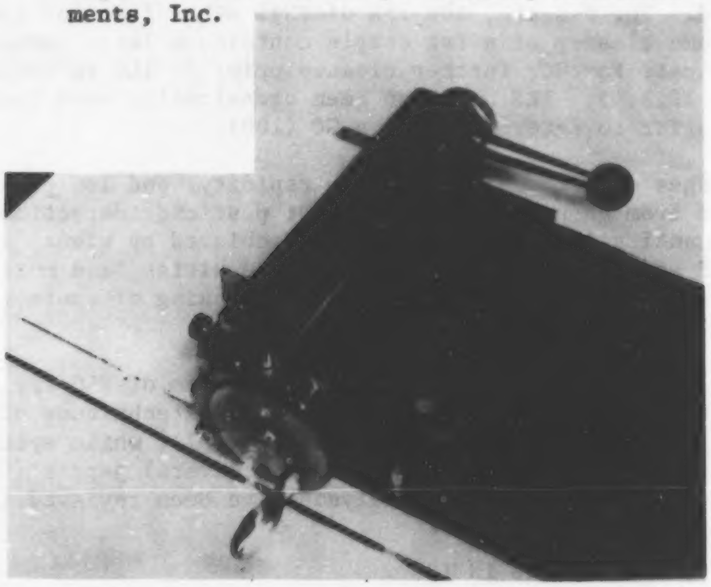
7K PRACTICAL CONSIDERATIONS IN TLC

Spots are applied to the thin layer using simple disposable capillaries, GC syringes, or automatic multiple spotting devices. All initial zones should be of small, uniform size, and only enough pesticide is spotted to allow for detection after the run. Care should be taken that the spotting pipet does not penetrate the surface layer. Standard solutions must be spotted on the same plate as the sample, preferably on both sides of the sample spot.

Layers are hand-coated with a commercial adjustable spreading device (Figure 7-B) or purchased pre-coated on glass, plastic, or aluminum backing. Analytical layers are usually 250 µm thick. Pre-coated layers are of high purity and uniformity and are used almost exclusively in most laboratories, especially for in situ quantitation by densitometry. Substitution of one brand of adsorbent for another or pre-coated for hand-coated plates often cannot be directly made. For example, silica gels with differing polarities or surface hardness (binders) may require modified solvent systems or detection reagents if similar results are to be obtained. Pre-coated silica gel plates, especially those prepared with an organic binder, are generally used as received from the manufacturer without activation.

Silica gel and alumina layers usually give the best results, but polyamide, microcrystalline cellulose, kieselguhr, zinc carbonate (105), and magnesium oxide, among other adsorbents, have also been used. For reversed phase TLC, hydrophobic C_{18} chemically bonded silica gel plates are commercially available.

Figure 7-B Desaga/Brinkmann Adjustable Applicator For Coating Regular or Gradient Layer Plates, Brinkmann Instruments, Inc.

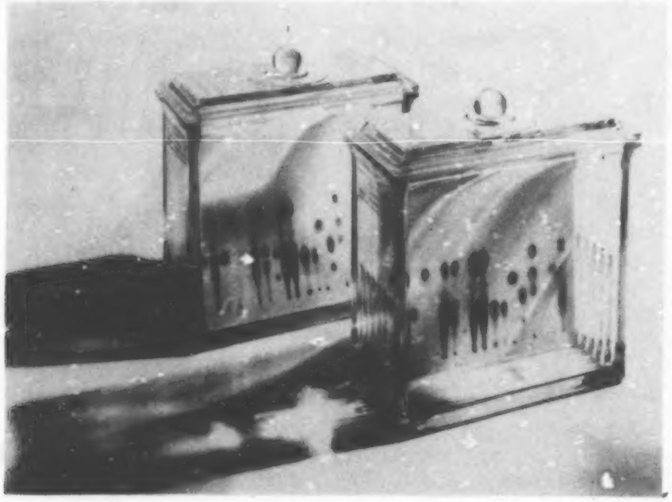


Chromatography is carried out in a development chamber, most often a rectangular, glass, paper-lined tank saturated with solvent vapors (Figure 7-C). Low volume "sandwich" chambers are also used. Both saturated and unsaturated atmospheres have been used to advantage and should be tested for optimum results in any particular application. Ascending development for a distance of 10-20 cm is typical. It is important to follow exactly all stated conditions when attempting to reproduce a separation. The temperature, development chamber design and equilibration, and water content of the adsorbent are probably the most frequent sources of variation among laboratories.

The technique termed "high performance thin layer chromatography" (HPTLC) has become increasingly important for separations and in situ quantitative analysis in the recent past. HPTLC is carried out on 10 x 10 cm, 7.5 x 7.5 cm, or 5 x 5 cm pre-coated layers of silica gel with a smaller particle size and a narrower particle size distribution than in conventional TLC plates, thereby giving improved resolution and sensitivity of detection. Volumes no larger than 1 μl must be spotted for these advantages to be fully realized. For manual application, spotting is usually done with a Pt-Ir tipped Nanopipet (or equivalent), or this type of pipet is used with an automatic spotting device that controls both the pressure of the pipet tip on the layer and the duration of contact. Solvent development is carried out in a miniature glass rectangular chamber or in a commercial, automatic U-chamber device producing radial zones (106) (a special radial scanner is needed to quantitate these separations). High resolution is achieved rapidly with short development distances (i.e., 5 cm or less).

In a typical residue analysis, it is virtually impossible to apply the whole cleaned-up sample extract or an appropriate, accurate aliquot as a spot of 1 µl or less, so HPTLC has not yet been widely used for actual samples. New approaches are appearing that may solve this problem by allowing a larger sample to be applied without sacrificing the benefits of the HP layer. One proposed solution utilizes a two-section plate with a high performance analytical layer above a spotting region; initial development concentrates the diffusely applied sample into a narrow zone at the interface of these layers (107). Another possibility is the use of programmed multiple development (apparatus from Regis Chemical Co.), which causes large initial spots to be narrowed during migration on the HP layer (108). HPTLC plates are available from Merck and Whatman, and HPTLC equipment from Camag, Inc. and Fotodyne Corp.

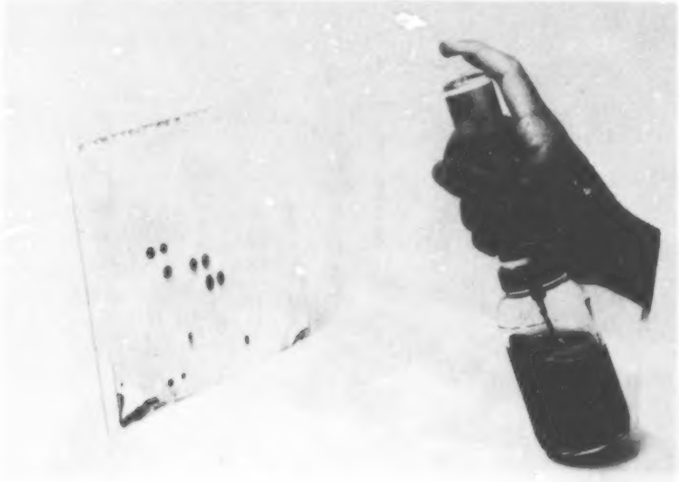
Figure 7-C. Desaga Rectangular TLC Tanks, Brinkmann Instruments, Inc.



The following solvent systems have proved to be generally useful for separation of a wide range of pesticides on silica gel thin layers: benzene mixed with varying amounts of ethanol for polar compounds or with hexane for those which are less polar; and a mixture of hydrocarbon plus acetone plus chloroform, with the addition of methanol for more polar pesticides. Examples include pentane-acetone-chloroform (65:30:5 v/v) or pentane-acetone-methanol-chloroform (70:15:10:5 v/v). The purpose of the chloroform is to control the evaporation of acetone in the atmosphere of an unsaturated tank. Proportions of the components are changed to suit the requirements of specific separations.

After development and air drying of the layer, spot detection may be achieved in a number of ways. Few pesticides are naturally colored, but colored derivatives may be made prior to spotting, e.g., dyes formed from aromatic amine moieties of urea herbicides by coupling with N-ethyl-1-naphthylamine (FDA PAM, Vol. II, Sec. 120.216). Colorless spots can be detected by applying a chromogenic reagent, either by spraying or dipping. A commercial aerosol spray device is shown in Figure 7-D. Dipping is the preferred method of application, if feasible, because of the uniformity achieved and the hazards involved in careless spraying of corrosive, toxic, or carcinogenic reagents. A Thomas-Mitchell dipping tank is recommended. Sometimes the reagent can be incorporated in the layer prior to development or included in the developing solvent. Naturally fluorescent spots can be detected under short (254 nm) or long (366 nm) wave UV light, or fluorescence may be induced by application of fluorogenic reagents after development or preparation of fluorescent derivatives (e.g., dansyl compounds) prior to spotting (109). Spots that absorb UV light are detected as quenched (dark) spots on layers containing phosphor activated by UV light (usually 254 nm). Radioactive (labeled) pesticides are detected by autoradiography and some fungicides are detected by direct bioautography.

Figure 7-D. TLC Aerosol Sprayer, Brinkmann Instruments, Inc.



7L QUANTITATIVE TLC

Quantitation of separated spots may be achieved by "eyeball" comparison between sample and standard spots run on the same plate or by some independent analytical method (e.g., spectrophotometry or GC) after scraping the spot, collecting, and eluting the pesticide from the adsorbent. Manual elution is simply carried out by scraping the area containing the pesticide spot, collecting the scrapings in a vial or tube, adding solvent and agitating (vortexing), filtering the adsorbent, and concentrating the filtrate containing the pesticide. An automated elution system is available from Camag, Inc. (110). Radioactive spots can be quantitated by scintillation counting after scraping or by automatic scanning of radioactivity on the layer.

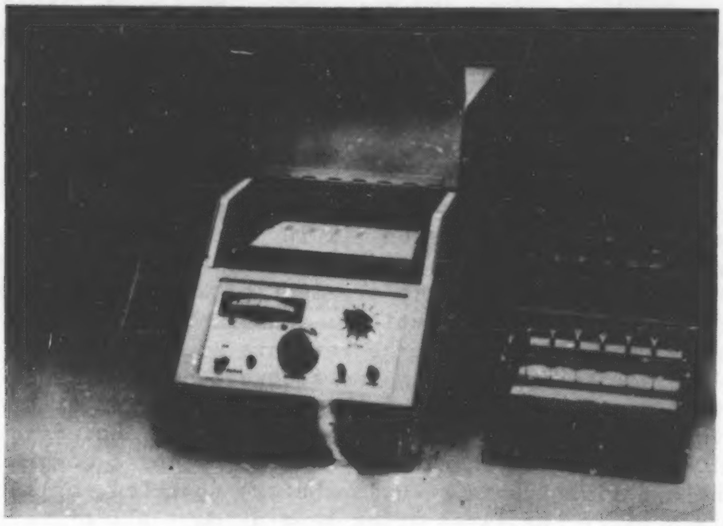
Colored, fluorescent, or quenched spots may be scanned on the layer when a spectrodensitometer is available. Quantitation is achieved by scanning sample and standard spots in the optimum instrumental mode and treating the resultant peaks, representing the amount of light absorbed or emitted, in the same manner as GC peaks for calculations. A versatile densitometer is capable of scanning in single or double beam and reflectance or transmission modes, and has monochrometers or filters for selection of the best wavelengths of incident and emitted (for fluorescence) energy. Important considerations for densitometry are adequate extract cleanup (111), precise and accurate spotting, uniform layers, R_F values between 0.3 and 0.7, uniform application of detection reagents, and optimum use of a good densitometer.

Fluorescence densitometry has proven to be the most advantageous mode for pesticide analysis in terms of sensitivity and selectivity. If the compound is naturally fluorescent (e.g., benomyl, Maretin, quinomethionate), the procedure is usually straightforward and measurements can be made immediately after separation. Sensitivity and reproducibility are usually very high because no reagents are added or sprayed on the chromatogram to produce background fluorescence. For the majority of pesticides that are not fluorescent, however, some kind of treatment is required. Possibilities for producing fluorescence include treatment of the plate with heat, acid, base, inorganic salts, or a combination of these; preparing a derivative in solution before spotting; or applying a fluorogenic reagent to the layer after separation. All of these options are included in the papers cited in Table 7-2.

Manual spotting is best performed with 1 or 2 µl Microcap disposable pipets, using repeated spotting with drying in between for larger volumes. It has been shown that sample delivery errors below 1% are feasible with Microcaps (112). Larger volumes of sample extracts are conveniently and reproducibly spotted with a device such as the Kontes automatic spotter that applies milliliter volumes of one to six samples or standards in small, uniform zones with little operator attention. Solutions are loaded into 5 ml capacity glass tubes and are delivered onto the layer through Teflon coated needles, the rate of flow and spot size being controlled by a stream of nitrogen or air focused onto the spotting location. The Kontes spotter is pictured in Figure 7-L and described in reference (113). Manual spotting of larger volumes onto commercial plates with an inert preadsorbent spotting area is quickly and reproducibly done with a Drummond digital microdispenser. Samples and standards, including total extracts, so applied are narrowed to a common, small initial zone size at the silica gel interface (114).

Pre-coated layers are recommended for quantitative TLC since it is very difficult to hand-coat layers with adequate uniformity. They are generally purified before use by a predevelopment with chloroform-methanol (1:1 v/v) followed by evaporation of the solvent in a dust free atmosphere. Uniform application of chromogenic or fluorogenic reagents is better achieved by dipping than by spraying. However, dipping is not always possible; its use depends on the reagent solvent, adsorbent, and type of compounds on the layer.

Figure 7-E. Fiber Optics Thin Layer Scanner and Automatic Spot Applicator. Kontes Glass Company, Inc.



When a new densitometric method is developed, the spots of interest should be scanned in all possible modes and directions and at a variety of wavelengths in order to obtain the best signal to noise ratios and selectivities. The optimum conditions are then used to obtain the calibration curve (linear range) and perform the analysis. Samples and bracketing standards should always be chromatographed on the same plate.

Thin layer densitometry is capable of precision of 1-2% on a routine basis and can rival GC and HPLC for determination of certain pesticide residues in the hands of an experienced operator. A book covering the principles and experimental details of thin layer densitometry, including a chapter on pesticide analysis, has been published (115). Table 7-2 contains some recent, selected applications of thin layer densitometry. A fiber optics scanner specifically designed for pesticide analysis (116) is available from Kontes (Figure 7-E).

TABLE 7-2 PESTICIDES QUANTITATED BY THIN LAYER DENSITOMETRY*

Compounds	Sample matrix	Scanning mode	Detection method	Reference
Acidic herbicides	standards only	fluorescence	4-bromoethyl-7-methoxycoumarin	(125)
Bayrusil	foods	fluorescence	heating	(134)
Benomyl	cucumber	fluorescence	--	(144)
Benomyl, carbendazim, and 2-AB	fruits, vegetables	quench	fluorescent layers	(118)
Captan, captafol	apple, potato	fluorescence	$NaClO_3$	(117)
Carbaryl	potato	visible	p-nitrobenzenediazonium fluoborate	(135)
Carbaryl	apples, water, lettuce	visible	p-nitrobenzenediazonium fluoborate	(142)
Chloramben	bean, tomato	visible	Bratton-Marshall reagent	(133)
Chlorophenoxy acid herbicides	water	visible	$AgNO_3$	(122)
Coumaphos	water	fluorescence	heating	(127)
Coumaphos	water	fluorescence	heating	(141)
Coumaphos and O-analog	eggs	fluorescence	heating	(131)
DDT	water	visible	$AgNO_3$	(135)
DDT	water	visible	$AgNO_3$	(137)
Fenitrothion	water	fluorescence	$SnCl_2$/fluorescamine	(121)
Fenitrothion, breakdown products, and related compounds	standards	fluorescence	fluorescamine	(145)
Gibberellins A_4A_7	apple pulp	fluorescence	H_2SO_4	(140)

TABLE 7-2 (Continued)

Compounds	Sample matrix	Scanning mode	Detection method	Reference
Glyphosate (via N-nitroso derivative)	shoots and roots	fluorescence	fluorescamine	(138)
Herbicides containing NH_2 or OH groups	water, soil	fluorescence	dansyl chloride	(119)
Maretin	milk, eggs	fluorescence	--	(129)
MCPA and Terbicil	apples	UV absorbance	--	(143)
OCl pesticides	human autopsy samples	visible	$AgNO_3$	(123)
OP insecticides	standards only	visible	$AgNO_3$ or enzyme inhibition	(126)
OP pesticides	water	fluorescence	hydrolysis/dansyl chloride	(128)
OP pesticides	tissues	visible	palladium chloride	(124)
Quinomethionate	crops	fluorescence	--	(130)
Thiabendazole	fruits	fluorescence	--	(132)
Thiourea	citrus fruits	UV absorbance	--	(136)
s-Triazines	standards only	quench	fluorescent layers	(120)
Triazines	water	visible	iodine	(139)

*Earlier analyses are reviewed by J. D. MacNeil and R. W. Frei in J. Chromatogr. Sci., 13, 279 (1975).

7M THIN LAYER SYSTEMS

a. Chlorinated Pesticides

Extracts of fatty and nonfatty foods cleaned-up on a Florisil column are chromatographed on prewashed alumina layers developed with heptane (for the 6% diethyl ether-petroleum ether Florisil eluate) or 2% acetone in heptane (15% diethyl ether fraction). Detection is provided by spraying with $AgNO_3$-2-phenoxyethanol reagent in ethanol or acetone and exposing to high intensity short-wave UV light to produce brown to purplish-black spots. The construction of a UV light apparatus containing four 15 watt lamps for rapid color development and allowing a variable distance between the TLC plate and the light source is described in the Canadian PAM, Section 14.10. Thin layer media must be very low in chlorine content, and other precautions and care must be taken to prevent large areas of the plate from turning brown or gray, thereby reducing the contrast of the spots with background. A sensitivity in the 5-500 ng range is possible with $AgNO_3$ reagent, with a light steaming before spraying often aiding the detection. Conventional 20 cm x 20 cm glass plates, commercial pre-coated TLC sheets, or 3-1/4 inch by 4 inch microslides may be employed. Complete details of these methods plus R_F values for numerous compounds in the aforementioned two solvent systems, as well as for an alternative system consisting of immobile dimethylformamide on alumina and isooctane as the developing solvent, are given in Sections 410, 411, and 413 of the FDA PAM. Silver nitrate has been incorporated into acid-washed alumina before the plates are coated so that only exposure to UV light is required for spot visualization (FDA PAM, Section 412). The $AgNO_3$ detection method has recently been studied in detail for the determination of chlorinated insecticides and herbicides (146).

Similar TLC procedures are described in detail in the EPA PAM, Section 12,B for the determination of chlorinated pesticide residues in serum and adipose tissue. An extract from 50 g of serum, cleaned-up on Florisil and concentrated to 100 µl before spotting, will produce a visible spot at 2 ppb, assuming that 10 ng of pesticide is detectable. An adipose tissue extract from a 5 g sample, concentrated to 500 µl, will give a readable spot at 10 ppb. The method involves TLC of the 6% and 15% Florisil column eluates as above, with additional prior cleanup of the 15% fraction on an alumina micro-plate developed with acetonitrile.

Silica gel layers developed with hexane, 1% acetone-hexane, 10-50% benzene-hexane, or 1% ethanol-hexane are recommended for screening chlorinated pesticides in foods at 0.1 ppm levels (Canadian PAM, Procedures 9.1 and 12.4). Complete details of plate preparation, extract concentration, and visualization with silver nitrate reagent are given in this source, along with figures of spot locations for eleven common pesticides in four mobile solvents.

For complex pesticide mixtures, two dimensional or multiple development techniques may be helpful. The former was used to identify organochlorine pesticides in blood and tissues (147) and the latter (148) for the separation of 13 common pesticides.

Extensive listings of additional solvent systems, corresponding R_F values, and detection reagents for chlorinated pesticides will be found in references (103, 123, 149, and 150). In reference (123), 26 solvent systems and 14 chromogenic reagents are evaluated for the determination of 12 organochlorine pesticides in blood, urine, and tissue samples.

b. Organophosphorus (OP) Pesticides

Cleaned-up extracts may be developed with methylcyclohexane on DMF-coated alumina layers and detection made by spraying with tetrabromophenolphthalein ethyl ester, $AgNO_3$, and citric acid. This reagent reacts only with thiophosphoryl compounds to give blue or magenta spots (FDA PAM, Section 431; EPA PAM, Section 12,B). Thio and nonthio organophosphates are developed on silica gel layers with isooctane-acetone-chloroform (70:25:5 v/v) and detected as blue or magenta spots by treatment with *p*-nitrobenzyl pyridine and tetraethylpentamine spray (FDA PAM, Section 432).

A two dimensional procedure (FDA PAM, Section 614.11; 151) has the significant advantage of specificity, obtained by bromine vapor oxidation of the OP pesticides before development in the second direction. Silica gel layers with toluene, 25 percent heptane in ethyl acetate, or ethyl acetate as developing solvents were used along with the Storherr charcoal column cleanup procedure and enzymatic detection with commercial horse serum cholinesterase and indoxyl acetate to identify 18 pesticides in crops at 0.01 ppm levels. A sandwich type chamber is specified for development to obtain the requisite resolution and sensitivity. The same procedure should be well suited to OP pesticides in human and environmental samples after appropriate cleanup.

Enzyme inhibition techniques are important for the selective and sensitive (pg-ng amounts) detection of enzyme inhibitors such as OP and carbamate insecticides and metabolites. These compounds inhibit esterases and thereby prevent hydrolysis of a chromogenic substrate. Procedures include separation by TLC (on silica gel layers sometimes as thick as 450 µm), optional treatment with bromine vapor or UV light, and spraying of the layer with enzyme and substrate solutions. Areas corresponding to inhibitors are visible as white spots on an intensely colored background; i.e., inhibited enzyme is surrounded by enzyme free to hydrolyze the substrate and thus produce color. While many OP pesticides are inhibitors *per se*, bromine or UV treatment is required to convert others to active inhibitors. For carbamates, UV or bromine treatment may produce no change or increased or decreased inhibition, depending on the compound. Sample extracts often require minimal cleanup prior to TLC analysis with enzymatic detection; for example, hexane extracts of many foods can be directly chromatographed. Section 9.2 of the Canadian PAM provides procedural details, tables of sensitivities and effects of bromine and UV treatment for OP and carbamate pesticides, and diagrams of mobilities with hexane-acetone (8:2 v/v), a generally useful development solvent for TLC on 450 μm silica gel layers. The preparation of these layers is detailed in Section 12.4 of the Canadian PAM. Several different esterases have been compared for the detection of 65 OP and carbamate pesticides in vegetables and fruits (152).

TLC enzyme inhibition methods and applications to pesticides have been reviewed (153-155) as have the merits of TLC for analysis of residues (156). The separation and detection of 42 phosphate compounds using five ternary solvent systems on three adsorbents and three selective chromogenic sprays have been reported (157). Twenty five solvent systems and several visualization reagents were evaluated for detection of 12 OP insecticides in tissues (124).

c. Chlorophenoxy Acid Herbicides

Extracts containing methylated chlorophenoxy acids are cleaned-up on a Florisil column and chromatographed on alumina layers using hexane saturated with acetonitrile as the developing solvent. Cleaned-up extracts containing free acids are developed for a distance of 3.5 cm on a pre-coated silica gel sheet with cyclohexane-acetic acid (10:1 v/v), then the sheet is dried and developed for 15 cm in the same direction with benzene-petroleum ether (3:1 v/v). Spraying with silver nitrate chromogenic reagent produces black spots with a sensitivity of ca 50 ng for the esters and 100-500 ng for the free acids. Details of both methods and R_F values are given in Sections 421 and 422 of the FDA PAM. Other detection reagents for these pesticides include Rhodamine B and Bromocresol green indicators (158).

d. Other Pesticide Classes

The TLC of other classes of pesticides including carbamates, ureas, phenols, dithiocarbamates, triazines, and organomercurials was reviewed in references (103) and (104). Applications, solvent systems, detection, and quantitation are covered in these references. TLC is particularly applicable to herbicides, many of which are polar and not susceptible to gas chromatographic analysis without derivative formation. Studies have been reported for the TLC of triazine herbicides on silica gel (120, 159) and polyamide (160); determination of 11 urea herbicides in water (161); detection of dithiocarbamate fungicides with congo red (162); separation of carbamate and phenylurea pesticides on polyamide (163); comparison of six reagents for detection of carbamate and phenylurea pesticides on HPTLC plates (164); and separation and identification of carbamate pesticides in post mortem material (165).

The TLC of five dithiocarbamate residues in chloroform extracts of leaves is detailed in Section 9.3 of the Canadian PAM. Silica gel layers developed with benzene for dimethyldithiocarbamates or acetic acid-methanol-benzene (1:2:12 v/v) for ethylenebisdithiocarbamates are used, with detection as yellow, brown, or green spots after a cupric chloride-hydroxylamine hydrochloride spray.

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Section 8

SAMPLING, EXTRACTION, AND CONCENTRATION PROCEDURES IN PESTICIDE ANALYSIS

This section treats a number of miscellaneous topics important in residue analysis. These include general considerations for collection and extraction of extracts. Specific procedures for extraction and cleanup of pesticide and metabolite residues are discussed in Section 9.

SAMPLE COLLECTION, PREPARATION, AND STORAGE

8A GENERAL CONSIDERATIONS IN SAMPLING

Special considerations must be given to the procurement, storage, and transportation of samples to be analyzed for pesticide residues. Procedures should ensure, as well as possible, that the pesticides originally present have not undergone degradation or concentration and that potentially interfering impurities have not been added. Plastics must be rigidly avoided as containers for samples to be examined by electron capture GC because minute traces of materials such as polyethylene may produce spurious responses. Similarly, metal containers may contain trace impurities such as oil films, lacquers, or rosin from soldered joints that will cause interference in GC analysis. In general, glass jars or bottles with aluminum foil or Teflon-lined lids are the most suitable sample containers, although it is sometimes possible for pesticides in stored extracts to be adsorbed onto the glass surfaces. Glass containers should be carefully precleaned as outlined in Subsection 3L in Section 3. Aluminum foil can be cleaned by agitating it in analytical reagent grade acetone followed by several rinsings with pesticide grade ethyl acetate and hexane. Plastic containers may be used, if necessary, only when non-interference with the subsequent analysis has been proved at its limit of detection. Important variables in the sampling and storage processes include the size of the sample, source, stability, contamination, intended use, behavior of the pesticides, and the temperature and time of storage.

Readers interested in a more exhaustive discussion of sampling and storage procedures than provided in the following sections of this chapter are referred to the publication "Guidelines on Sampling and Statistical Methodologies for Ambient Pesticide Monitoring" (Monitoring Panel, Federal Working Group on Pesticide Management, Washington, DC, 1974). This 60-page manual contains chapters on statistics and study design, air, soil, the hydrologic environment, estuaries, fresh water fish, wildlife, foods and feeds, and human tissues. The 20-page booklet entitled "Guidelines for Sampling and Transporting Samples for Pesticide Residue Analysis" (Federal Interdepartmental Committee on Pesticides Check Sample Program, London, Ontario, Canada, April, 1979) contains detailed information on dry feeds, plants and soils, food products, wildlife, tissues, forest substrates, water, and fish. The influence of sampling methods on residue analytical results, sampling criteria, and statistics of sampling data have been described (1).

8B REPRESENTATIVE VS. BIASED SAMPLING

Samples collected for the purpose of assessing tolerance infringements, such as with agricultural and food products, should be random and representative. To the contrary, most environmental samples are deliberately chosen to be biased in nature. For example, a sample of water to be analyzed for the highest possible pollution in a stream or lake would best be taken as a grab sample from the point of maximum pollution introduction (such as an effluent pipe from a factory) rather than from the center of the river where it might be most representative. If, on the other hand, the objective is an average residue profile of the entire body of water, the final sample would preferably be a composite of a number of subsamples taken at various locations and water depths. Analysis of a sick bird or fish in the middle of a metabolic cycle would usually be more useful for determining any pesticide contamination than a dead specimen that is likely to contain only metabolites. Similarly, human stomach washings (lavage) taken at an early stage are more likely to contain parent pesticides and to be useful for indication of pesticide poisoning.

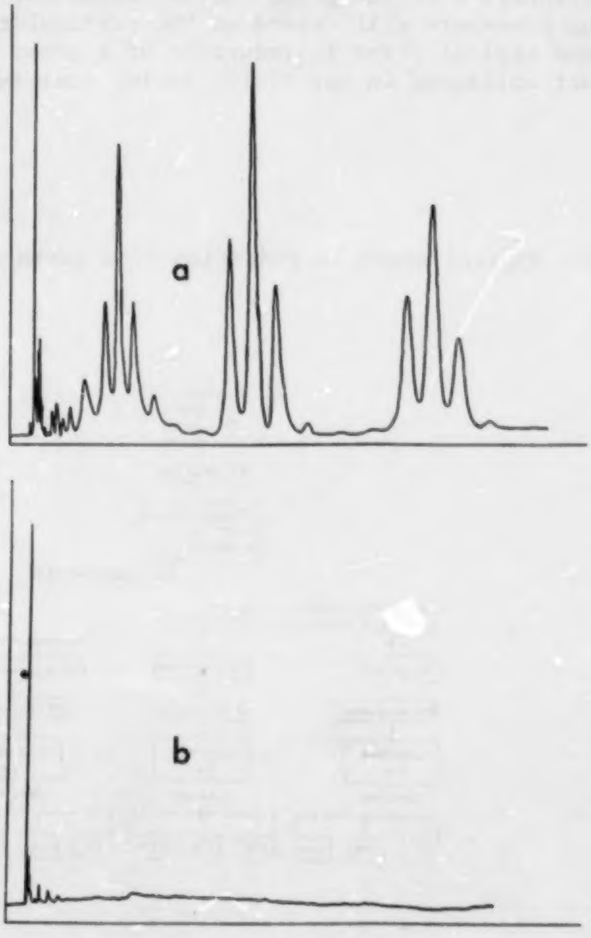
It is important that the analyst be aware of these considerations and that he be consulted when the sample to be collected is decided so that it is valid for the purpose of the analysis and valuable time is not wasted on a worthless sample.

8C SAMPLE CONTAINERS

Section 2 of the EPA Pesticide Analytical Manual specifies suitable sample containers for various sample types. These include wide mouth glass bottles with Teflon or aluminum foil lined screw caps for autopsy tissue samples of less than 25 g, glass vials of at least 7 ml capacity for blood (avoid rubber or cork caps), empty pesticide-grade solvent bottles for water samples, and pint or quart capacity mason jars for larger environmental or agricultural samples. Sample collection glassware should be scrupulously cleaned as outlined in Section 3 of this Manual. Special precautions must be taken in preparing glass containers and caps and taking samples for PCP analyses because of the ubiquity of the chemical. These are outlined in Section 5,A,(4),(a),IV of the EPA PAM. Specimens intended for organochlorine compound analysis are never wrapped directly in paper, cardboard, or plastic.

It is common practice in some laboratories to wrap tissue or other samples in aluminum foil prior to analysis. Figure 8-A, part a, shows a gas chromatogram of a pentane rinse of the shiny side of commercially available aluminum foil. The amount of rinse injected corresponded to 2 sq. cm of foil. The GC conditions included the use of a 10% OV-210 on Gas-Chrom Q column and a ^{63}Ni EC detector, typical of those used for analysis of pesticides and PCBs. Part b shows the corresponding rinse of the dull side of the same foil. In general, the amount of interfering material was found to vary with the brand and lot of foil. However, the risk of contamination from this source dictates that aluminum foil not be used for packaging samples without a thorough acetone prerinse (2).

Figure 8-A. Gas chromatogram of pentane rinse of aluminum foil on OV-210 column with ^{63}Ni electron capture detector. Amount injected corresponds to 2 sq. cm of foil. a = shiny side of foil; b = dull side of foil (2).

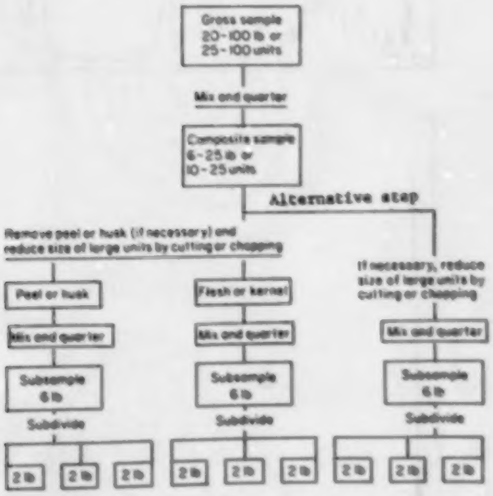


It is good procedure to clearly label collected samples with all pertinent information such as a code number, date and time of collection, type of sample, place and method of collection, description of collection site, size of sample, etc. All samples that are perishable are shipped to the laboratory in styrofoam containers with dry ice. A detailed description of systematic procedures used for receiving, numbering, and storing environmental samples at the National Monitoring and Residue Analysis Laboratory, Gulfport, MS, has been published (3). A strategy for documenting the chain of custody of samples that has the potential for being used as evidence in a legal proceeding or agency enforcement action is detailed in Section IV of the EPA National Enforcement Investigations Center Pesticide Product Laboratory Procedures Manual (see Section 3E of this Manual).

8D SAMPLE COMPOSITING

After collection of a valid gross sample, compositing or reduction to an analytical size sample may be required, especially for agricultural and food samples. The general requirement is that the small analytical sample must be fully representative of the gross sample collected. The exact steps in the compositing procedure will depend on the particular sample involved. Figure 8-B shows typical steps in reduction of a gross sample of an agricultural product collected in the field, during processing, or at the market.

Figure 8-B. Typical steps in reduction of a gross sample


Gross sample
20-100 lb or
25-100 units
Mix and quarter
Composite sample
6-25 lb or
10-25 units
Alternative step
Remove peel or husk (if necessary) and reduce size of large units by cutting or chopping
If necessary, reduce size of large units by cutting or chopping
Peel or husk
Flesh or kernel
Mix and quarter
Mix and quarter
Mix and quarter
Subsample 6 lb
Subsample 6 lb
Subsample 6 lb
Subdivide
Subdivide
Subdivide
2 lb
2 lb
2 lb
2 lb
2 lb
2 lb
2 lb
2 lb
2 lb

8E STORAGE OF SAMPLES

As a general rule, samples should be analyzed as soon as possible after their collection. If storage is necessary, it should be under prescribed conditions that preserve the integrity of the original sample. Samples other than water are ordinarily stored in a freezer below 0°C, but, even then, physical and chemical changes may occur in either the sample or in the residues sought. Because many pesticides are photodegradable, it is advisable to protect samples and any solutions or extracts from needless exposure to light.

Tissue samples that are to be extracted within 24 hours may be held at normal refrigerator temperature (+2 to +4°C). If extraction is not to be carried out within this time, the samples should be deep frozen at -12 to -18°C. If tissues are stored in a "self-defrosting" freezer in unsealed containers, the weight can markedly decrease due to desiccation. If the tissues were not weighed prior to freezing, or if they are to be subdivided at a later time, this desiccation may make it impossible to relate the amount of substance determined analytically to its original concentration in the tissue (2). A related problem occurs when samples experience repeated freezing and thawing. Adipose tissue in particular has a tendency to "leak" lipid when the cell membranes are disrupted by a freeze-thaw cycle. In a series of experiments in which such cycles were deliberately applied to a collection of samples of adipose tissue from a rat, the apparent lipid content of the tissue (mg per gram of tissue) decreased by an average of 10% after three freezings. This loss was only apparent, and was not observed if the tissue was extracted in the original storage container (2).

Blood samples that are to be separated for subsequent analysis of the serum should be centrifuged as soon as possible after drawing. If the serum is to be analyzed within a 3-day period, storage at +2 to +4°C is suitable. If storage is to be for longer periods, it is preferable to deep freeze at -12 to -18°C. Otherwise, DDT may degrade in contact with broken red blood cells (hemoglobin).

Agricultural or environmental samples that are to be analyzed for organophosphates should be placed in tight containers and stored in deep freeze as soon as possible after sampling unless sample preparation is to be conducted within a very few hours. No difference was found in measured residue levels for a series of OP pesticides when food samples were extracted immediately or after storage at -17°C for several months (4).

Water samples should be extracted at once, if all all possible, or stored in the dark at 4°C to avoid rupture of the container as a result of freezing. Pesticides can be adsorbed on the glass container during storage, so the container should be rinsed with solvent if the extraction is not made in the container itself. For carbamates, the sample is acidified immediately after collection with sulfuric acid and 10 g of sodium hydroxide are added for each liter of sample. Maximum storage is 24 hours for all compounds except chlorinated hydrocarbons, which can be held for up to 30 days.

Whole fish can be stored for up to six months if an even temperature of at least -26°C is maintained with a good glaze on the sample and rapid initial freezing. Homogenized samples require less storage space, but these samples should be monitored for stability of the compounds of interest if held longer than one month.

If lengthy storage is required prior to analysis, a good alternative to storage of sample is to extract the sample at once, remove most or all of the solvent, and store the extract at a low temperature. Decomposition in samples that must be stored can be evaluated by storing spiked controls along with the samples. Organophosphorus pesticides field-extracted with chloroform from water were successfully preserved for three weeks upon refrigeration. Of the 16 compounds tested, only EPN and malathion were not stable (5).

If freezing is not possible, wildlife and fish samples may be preserved in formalin or alcohol. Because analytical results are usually in terms of wet weight, the wet or "fresh" weight of the sample before it was preserved should be recorded, as well as the volume of preservative used in each jar. Specimens preserved in formalin or alcohol must be accompanied by a "control" jar. This jar must contain the same mixture used in preserving the specimens, and must be prepared (i.e., rinsed and sealed) in the same manner as the jars containing specimens. This may not be equivalent to freezing for storage of samples, however. For example, Abate was partially converted to Abate sulfoxide in fresh samples stored in formalin or formalin plus 5% acetic acid, but not in frozen samples (6). Formaldehyde should be checked for the presence of PCB contamination prior to use as a sample preservative (7).

Comments pertinent to collecting samples of different types will be presented in the Subsections 8F to 8K. Methods for the analysis of the various sample types are surveyed in Section 9 of this Manual.

8F SAMPLING OF AGRICULTURAL AND FOOD PRODUCTS

Procedures for sampling, sample preparation, sample compositing, and sample reporting, as required by Federal law, for all commodity types are outlined in detail in Sections 140-143 of the FDA Pesticide Analytical Manual, Volume I. Section 3 of the Canadian Department of National Health and Welfare Analytical Manual for Pesticide Residues in Foods gives guidelines for systematically obtaining representative samples of processed and packaged foods, bulk foods, and field crops and for handling, shipping, and storing samples. Recommended minimum sizes are tabulated for different samples, with a general sample requirement of n product units, where n equals the square root of the total but need not exceed 10-15 separate units.

Section 4 of the same Canadian PAM covers laboratory preparation of analytical samples from gross samples of fresh, frozen, and canned vegetables, fruits, and juices; dry cereal grains, flakes, dehydrated fruits and vegetables; animal tissues; eggs; butter and margarine; milk and cream; cheese and nuts; fats and oil; and fish and fish products.

It is suggested that readers interested in analysis of sample substrates of this type for legal compliance to tolerance levels should refer to these two excellent sources of information. Sampling methods for trace organic analysis of foods have also been described by Horwitz and Howard (8). If the purpose of an analysis is to obtain information on maximum residue levels in a particular situation, biased sampling would be used, e.g., the lower perimeter of fruit would be sampled from certain trees most likely to have received a higher dose of pesticide spray.

8G SAMPLING OF BIOLOGICAL MATERIALS

Adipose tissue, blood, and urine samples from live and autopsy animal and human subjects are commonly analyzed for pesticide residues. The amounts of sample required, the time of collection, and the compound to be detected are determined by the nature of the pesticide(s) of interest. Pesticides that degrade or are metabolized readily may be absent in a particular sample, but their original presence can be deduced by determination of metabolites such as alkyl phosphates from OP pesticides, phenols from chlorophenoxy acid herbicides or carbamate insecticides, or DDA from DDT. If body tissues or fluids are analyzed quickly in cases of high exposure, the chance of finding the parent pesticide is greatly enhanced. If exposure is low or a long time has elapsed after exposure, the analyst must be familiar with pesticide metabolism in order to choose appropriate samples and metabolites to determine. For example, the highest concentration of organophosphorus pesticide urinary metabolites will be found from four to eight hours after the donor's exposure (EPA PAM, Section 6,A,2,(a),V). When concentrations of pesticides or metabolites are expected to be small, samples must be larger, e.g., morning urine samples or 24-hour pooled specimens.

The majority of human adipose tissue samples are taken during autopsy by an attending physician. Samples should be placed in a clean glass container with a foil-lined (never rubber- or cardboard-lined) screw cap. The aluminum foil should be prerinsed with acetone. Plastic bags or bottles must be avoided since they can contribute traces of impurities such as phthalates to the sample, causing spurious GC peaks when the final concentrate is examined by EC-GC or GC-MS (EPA PAM, Section 5,A,(1),(a),V). Up to 2% of radiolabeled DDT was found to be lost by "extraction" into the plastic when liver samples were stored in polyethylene bottles at 4°C overnight. This radioactivity was not removed when the bottles were washed, so that the loss for one sample could constitute a contamination for the next sample stored in the same bottle (2).

Whole blood samples are transferred to glass vials with Teflon or foil lined screw caps, and the required serum aliquot is removed after a period of settling in a refrigerator and subsequent centrifugation. Serum is stored in a refrigerator at 2-5°C if the analysis is to be performed within 24 hours or in a deep freeze (-15 to -25°C) for longer storage periods. The analysis of chlorinated pesticides is not adversely affected by such storage for periods up to six months (EPA PAM, Section 5,A,(2),(a),IV).

8H AIR SAMPLING

The EPA has in the past operated a nationwide air monitoring program in order to gather information on the extent of human exposure to airborne pesticides. This program utilized Greenburgh-Smith impingers containing ethylene glycol for trapping organophosphorus and halogenated hydrocarbon insecticides both in the vapor phase and as dusts. The air was drawn through the impingers by means of a vacuum pump, the amount sampled (cu. m) being controlled by means of a flow meter and timer. The ethylene glycol sampling procedure did not prove acceptable in terms of convenience or reliability, and the EPA national air sampling program was discontinued. However, the ethylene glycol impinger continues to be used by some laboratories in local monitoring programs (3, 9, 10).

Robert G. Lewis of the U.S. EPA Health Research Effects Laboratory has written an extensive review of sampling methods for airborne pesticides (11). Included are discussions of many types of accumulative samplers (e.g., impactors, bubblers, liquids supported on solid substrates, polymer foams, etc.), reactive samplers, continuous and sequential samplers, and grab samplers. Recent reports of pesticide recovery from air have included the use of tubes containing XAD-2 resin for trapping 2,4-D acid and its ester and amide derivative with 86-96% efficiency (12); hexylene glycol contained in glass scrubbers for recovery of dieldrin and heptachlor at 0.1 ng/cu. m (13); and XAD-2 resin for organothiophosphates (14).

One of the most promising approaches to the sampling of air involves use of polyurethane foam. The updated review of air sampling methods in Section 8,A of the EPA PAM contains a discussion of this method in addition to other approaches and apparatus recommended by the EPA for high volume ambient air sampling, indoor air sampling, crop re-entry monitoring, and workplace and personnel monitoring. Polyurethane foam vapor traps following a particle filter have been evaluated (15) for sampling of pesticides, PCBs, and polychlorinated naphthalenes. Collection rates up to 360 cu. m of air per 24 hours and sensitivities as low as 1 cu. m for some compounds can be achieved. The filters and plugs were Soxhlet extracted with hexane-ethyl ether (95:5 v/v) at 4 cycles per hour for 16-24 hours, and OCl pesticides were determined by EC-GC after alumina column cleanup and OP pesticides by FPD-GC without cleanup. Collection was generally satisfactory but was poor for the more-volatile OCl compounds. Recovery was ca 75% for OP pesticides. It was shown that a second trap in series with the first did not necessarily improve recovery values. The collection of dieldrin, lindane, trifluralin, dacthal, chlordane, and heptachlor on polyurethane foam was studied and optimum plug size and shape for any chosen sampling rate were given. Trapping efficiency depended on pesticide vapor pressure and the flow rate of air. The quantitation limit was ca 0.1 ng/cu. m in a 5 cu. m air sample. It was crucial that the plugs were carefully protected from laboratory contamination and Soxhlet extracted with pesticide-grade acetone and hexane prior to use if clean blanks and highest sensitivity were to be achieved (16). Other collection efficiency data for pesticides and PCBs are included in Section 8,B of the EPA PAM.

Because of possible pesticide degradation, air sampling apparatus should be shielded from light during sample collection.

8I WATER SAMPLING

The design of a comprehensive pesticide sampling program for environmental waters is a specialized topic that is covered in publications available from the Water Quality Control Division of the USEPA, National Environmental Research Center, Cincinnati, Ohio. Important considerations include the objective of the study, frequency of sampling, location of sampling stations as related to hydrologic conditions, and the selection of sampling methods. The following is a brief review of some important selected factors in a sampling program.

a. Grab Samples

Water can be collected by taking one instantaneous ("grab") sample from a given location, directly filling the sample container. The usual technique is to submerge the container a few inches below the water surface during filling to avoid skimming off any floating film that would be least representative of the vertical water column. Several collections should be taken at various depths and locations to provide a more representative sample. Care should be exercised to avoid disturbing bottom sediment. Discrete samples from various depths can be obtained with standard samplers consisting of a metal outer container with a glass sample collection bottle inside (e.g., Precision and Esmarch samplers, EPA PAM, Section 10,A,II). Grab sampling is often sufficient for lakes, reservoirs, etc., that are not subject to rapid transitional changes.

Grab samples less than 2 liters are collected in wide mouth glass bottles, and samples of one gallon or more in the glass bottles in which pesticide quality solvents are supplied. All bottle caps should be Teflon lined. The sample size is dictated primarily by the expected residue levels, the sensitivity of the analysis, and the need to run duplicate, spiked, and background analyses. A 500-1000 ml sample may suffice from water where pesticide levels are expectedly high, while 2 liters or more may be needed for a surveillance program where no high levels are anticipated. Rainwater is collected in clean glass containers rather than metal or plastic. Samples should include information that will help the analyst choose a proper analytical method and interpret the results. This includes the location of sampling, depth, suspected contaminants, type of sample (surface water, waste discharge, etc.), and agricultural activity or spills in the immediate area or upstream.

Many pesticides are unstable in water, so samples should be analyzed as soon as possible after collection, ideally within a few hours. If this is impractical because of distance from the sampling site to the laboratory and/or the laboratory work load, store the sample in a refrigerator or freezer. Samples being examined solely for organochlorine residues may be held up to a week under refrigeration at 2 to 4°C with no adverse effect. Those samples to be analyzed for organophosphorus or carbamate pesticides should be frozen immediately after drawing the sample because of rapid degradation in aqueous media (Table 1, Section 10,A of the EPA PAM shows data for the degradation rate of 29 pesticides in water at ambient temperature in sealed containers (17)). pH adjustment is required for some samples immediately after collection (e.g., adjustment to pH 2 with sulfuric acid for phenoxy acid herbicides). Holding time and storage conditions must be reported along with the analytical results and corrections made if rates of pesticide degradation are known. Exposure of samples to sunlight should be avoided.

Every effort should be made to perform the solvent extraction step at the earliest possible time after sampling, irrespective of the classes of pesticides suspected of being present. Especially unstable pesticides can be extracted immediately in the field. The resulting extracts can be safely stored for periods up to three or four weeks at -15 to -20°C before proceeding with subsequent cleanup and determinative steps. One disadvantage of glass sample bottles is possible breakage in shipment, and care should be exercised in proper packaging to avoid this. Another disadvantage is the already-mentioned possibility of pesticide adsorption on glass surfaces. Reduced recovery (>90% to 46-68%) of DDT in water analysis upon storage has also been noted due to adsorption on suspended matter in the sample (18).

The assumption made is that a grab sample is at least representative of the immediate water mass from which it was taken and somewhat representative of the water that will pass the sampling point during some limited future time interval. The grab sample is amenable to use in both random and nonrandom sampling programs. The number, frequency, and distribution of samples collected will depend on the study objectives and the variability within the "population" being sampled.

After sampling, pesticides are extracted from water, cleaned up and concentrated as necessary, and determined by GC or an alternative method. Pesticides in clean water (e.g., drinking water) can be detected at 5-500 ppt levels by electron capture GC without the need for extensive extract cleanup. Impurities in "dirty" samples will require additional cleanup steps, and background problems will cause difficulty in analyzing these low levels accurately.

b. Continuous Samplers

Continuous and automatic devices are often used for sampling flowing bodies of water such as rivers and streams. Activated carbon filters have been widely used for adsorption of pesticides and other kinds of organics in natural waters since they were developed and introduced by the U.S. Public Health Service in 1951 (19). The technique involves passage of a continuous, constantly controlled volume of water through a column of activated carbon followed by desorption by means of elution or by Soxhlet extraction with a suitable solvent or combination of solvents. The variable efficiency and consistency of pesticide adsorption and desorption from the adsorbent prior to determination, ease of contamination with extraneous organic substances, and bacterial and oxidizing attack on the sorbed pesticides have caused problems with carbon columns (20, 21).

Filter materials which have been recommended as alternatives to carbon for collection of pesticides (usually chlorinated insecticides) from natural waters include reversed liquid-liquid partition systems (a hydrophobic phase coated on a support) and other adsorbents. Carbowax 4000 (5 g) and n-undecane (22), silicones chemically bonded to diatomaceous earth support (23), covalently bonded aromatic and alkyl chlorosilanes on Celite (24), porous polyurethane foam columns (for pesticides and PCBs) (25, 26), polyethylene film (20-25 μm thickness) (27), and polyurethane foam coated with selective adsorbents (28) have all been used with varying success.

The XAD macroreticular adsorbent resins (XAD-1, -2, -4, and -7) have been used to collect organics from both potable (29, 30) and sea (31) water. Optimum conditions for use with XAD-4 resin were found to be 2 g of adsorbent, a flow rate through the resin of 8 ml/minute, and 100 ml hexane-diethyl ether (10:1 v/v) as eluting solvent. Among 10 chlorinated insecticides studied, only aldrin and p,p'-DDE were not quantitatively recovered, and recovery of PCBs was 76% (32). Details for use of XAD-2 and -4 resins for many classes of trace organic water contaminants have been published (33) and recoveries between 81 and 96% were reported for 20 ppt levels of atrazine, lindane, dieldrin, DDT, and DDE (47% for aldrin). An EPA report (34) recommends XAD-2 resin for routine monitoring of sea water for chlorinated insecticides and PCBs. Average recovery for XAD-2 extraction of fortified natural waters collected across Canada was 85% for the 10-100 ng/liter levels of ten OCl pesticides (recovery of mirex was unacceptably low) and 82% for 250 ng/liter levels of PCBs; blanks from the resin were a low 4 ng PCBs/liter (35). Concentrations as low as 0.1 ppt of PCBs and organochlorinated pesticides were detected by recovery from water on small XAD-2 columns (36), and ng levels of carbamates were recovered (86-108% at 0.01-1 ppm levels) with the same adsorbent (37). Amberlite XAD-4, porous polyurethane foam, and undecane plus Carbowax 4000 on Chromosorb were comparable for extracting ten OCl insecticides from environmental water samples (22).

Continuous liquid-liquid extractors are an alternative to the filter-adsorbent processes preferred by some analysts. A multi-chamber extractor with internal solvent renewal replenishing (38) allowed extraction of 135 liters of water at rates of 0.5-1.0 liter/hour and recovered greater than 97% of ppb levels of pesticides. Subsequently, a similar modified apparatus permitted use of both heavier- and lighter-than-water solvents (39). A simple and rugged field version of the Kahn and Wayman apparatus (38) excluded solvent recycling and was based on mixed settling (40). This apparatus, which consisted of an extraction unit, magnetic stirrer, and pump, provided quantitative recovery of pesticides and PCBs at levels of 0.1-1.0 ng/liter of river water.

More recently, a similar *in situ* apparatus designed to solvent-extract large amounts of sea and river water continuously while situated at a desired depth at the sampling site has been described (41). A Teflon helix, continuous liquid-liquid extractor, plus a continuous evaporative concentrator recovered µg to ng per liter amounts of OP pesticides from river and sea water or from secondary sewage effluent with >80% efficiency (42). A comparative study of recoveries from river water by continuous extraction and activated carbon filters showed that the recoveries were similar but the former was less costly (43).

The theory for extracting chlorinated pesticides continuously from water with a stationary immiscible solvent is discussed in reference (40).

See Section 10,A,III-V of the EPA PAM for updated material on water sampling.

8J SAMPLING OF HOUSE DUST, SOIL, AND STREAM BOTTOM SEDIMENT

House dust is collected with a vacuum cleaner, air dried, and sieved prior to analysis. Soil is sampled by collecting cores or borings of a known diameter cut to a depth of ca. 3-4 inches or more from the centers of plots 1 sq. m in size. Ten to twenty cores representing a surface area of at least 200 sq. m are recommended. The first 2 inches of core, containing the grass or crop cover and roots, are separated from the underlying soil. Corings representing each layer of soil are combined, quartered, and divided into 2 lb samples for analysis. Soils are analyzed in an air-dry state after sieving to remove foreign material. Another reported procedure for soil sampling (3) involves collection of cores 1-3 inches deep and 3 inches in diameter with a hand-operated auger; on a 1/4 acre site (105 feet x 105 feet), sampling begins 7.5 feet from the border of the site, and a core is collected every 15 feet until 7 cores are obtained. The process is repeated along parallel lines separated by 15 feet from the original sampling line, until a total of 49 cores are collected. The cores are sieved through a hardware cloth screen into a 3 gallon galvanized pail and thoroughly mixed. The sample is transferred to two one-half gallon cans with lids for shipment to the laboratory. There is no way to collect a truly representative soil sample, and reproducibility of results on different samples taken from the same area is often expectedly poor.

Sediment from the bottom of a body of water provides information concerning the degree of pollution resulting from pesticides, particularly those that are not readily degradable. This information combined with residue data on the water and resident biological life gives an overall pesticide contamination profile of the body of water. Bottom sediment varies with respect to both particle size composition (surface adsorptive power) and organic content. Therefore, sample sites should be selected at random in an effort to collect samples representing a range of variation. In some cases consultation with an oceanographer can indicate where one would be likely to find the maximum amounts of pollution from considerations such as currents and industrial effluent discharges.

About a quart of sediment is a typical sample size. Actual collection is accomplished with one of a variety of core samplers or dredges. A diagram of a dredge-type device for collecting sediment samples has been published (3). The dredge is thrown into the water at least 10 times to collect samples, which are transferred each time to a galvanized pail. The total sample is mixed and transferred to one-half gallon cans (with a hole in each lid to release any gas buildup from organic matter in the sample) for shipment to the laboratory. A simple bottom sediment collector composed of a steel can attached to the end of an aluminum pole has also been described (44). Samples may be preserved with formalin or a variety of other sterilants provided they do not affect the analyses to be run. Samples are air dried and ground prior to analysis. They are stored, if necessary, in a freezer if volatile compounds such as 2,4-D ester may be present.

8K MARINE BIOLOGICAL AND WILDLIFE SAMPLES

A problem sometimes encountered when collecting plankton and bottom organisms is obtaining the minimum weight necessary for successful analysis.

As a general rule, a minimum of about 10 g will be required. Collected organisms can be frozen at once or preserved with 5-10% formalin or 70% ethanol, prepared with distilled water rather than the water from which the collection was made. This eliminates the possibility of pesticides in the water concentrating in the organisms over a period of time. Any added preservative must be extracted and analyzed to determine if exchange of pesticides from the organisms to the preservative has occurred.

Sufficient masses of plankton are collected by use of a tow net behind a boat or by pumping water through a net. Bottom fauna are collected with dredges or dip nets. Samples are washed through a screen and organisms are hand picked from the remaining debris.

Fish are collected utilizing seines, gill nets, traps, electrocution devices, otter trawls, or angling. Wrapping the fish in aluminum foil and preservation by quick freezing in dry ice is most desirable. When this is not possible, liquid preservatives are used. Larger fish should be injected with preservative from a syringe to prevent decomposition of internal organs. Fish stored in formalin plus 5% $Na_2S_2O_3$ showed no loss of Abate (temephos) residues (>1 ppb) for up to three weeks (45).

Fish can be analyzed whole to yield data on gross contamination, or the fish can be sectioned to obtain information on edible and non-edible parts. Analyses of individual organs and tissues yield information on distribution of pesticides in the fish. Analysis of blood from a dying fish may be valuable for determining probable cause of death where pesticide exposure is suspected. The blood is obtained by cutting the tail at the caudal peduncle and collecting and freezing the blood in a small vial.

Invertebrate samples are collected in pitfall traps, as described by Wojick et al. (46). Bird samples are collected using Japanese mist nets placed near a water source or in a cove where the net is not visible. Traps baited with peanut butter or some other foodstuff are employed for sampling mammals. These traps, which are available in a variety of sizes, have a trap door that closes when the animal enters to take the food. Non-crop vegetation samples are obtained with shears, sickles, pocket knives, etc., usually from the same sampling area as soil samples. All of these samples are sorted, wrapped in aluminum foil with the shiny side out, tagged, and placed in a plastic bag for shipping (3).

Some of the material in the sections on sampling was adapted from an EPA training course manual (47). A review that includes some of the above sampling procedures and additional methods for collection of environmental samples has been published (3).

8L CONTROL OF PROCEDURES FOR EXTRACTION OF RESIDUES

Specific procedures for the extraction and cleanup of pesticide multiresidues in many sample types are surveyed in Section 9 of this Manual. This subsection discusses general considerations of pesticide extraction from collected samples.

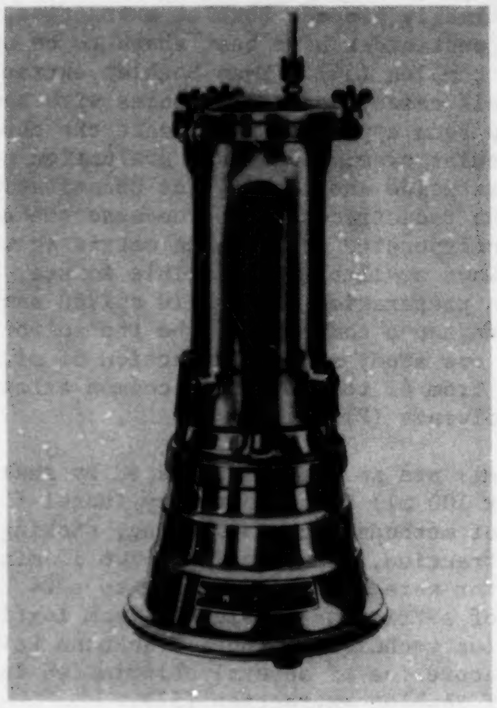
Many solvents are employed for extracting residues, depending on the polarity of the pesticide and the amount of co-extractives expected from the particular substrate. Solvents range from hexane or petroleum ether for nonpolar organochlorine and organophosphorus compounds to methylene chloride (dichloromethane) for polar carbamates. Chloroform, diethyl ether, ethyl acetate, benzene, acetonitrile, methanol, acetone, and various two- and three-component mixtures of these have all been widely used. Addition of acid to the organic solvents may aid extraction of acidic pesticides such as 2,4-D herbicide. Acetonitrile is an excellent general purpose extraction solvent for low fat-content samples (acetonitrile plus ca 35% water for low moisture samples), and hexane/acetonitrile systems are widely recommended for partition cleanup.

Although it has been shown in some cases that recovery of pesticides from tissues during extraction does not necessarily correlate with recovery of lipids, it is usually desirable to use an extraction solvent that will quantitatively extract lipids with the pollutants for reporting purposes (2). A study (48) has compared the recovery of lipid by nine solvent mixtures from human adipose tissue for pesticide determination. Extraction procedures should always be validated for each class of compounds in each type of sample matrix to which it is applied. In addition to the nature of the analyte, the toughness, water content, and lipid content of the sample matrix will influence the effectiveness of a given extraction procedure (2).

Different techniques are employed for bringing the extraction solvent and sample into contact. The best extraction is obtained, in general, by achieving the most intimate contact between the two, although the type of residue is an important distinction. When emulsions result from vigorous shaking or mixing during extraction procedures, centrifugation will usually be effective in separating solvent layers. A surface residue can usually be extracted by a simple washing procedure, while the more common internal residues can be extracted only after fine maceration of the sample. Some soil samples tenaciously bind pesticides and require long periods (e.g., 8 hours) of Soxhlet extraction rather than shorter periods of blending as is common with plant materials. Blending of the sample plus solvent in a Waring blender (Figure 8-C), Omni-Mixer, Wiley mill, or Hobart food chopper is probably the most usual extraction procedure in use today, especially for biological, plant, and food samples. Some additional sample subdivision, such as cutting, chopping, or grinding usually precedes the blending operation. A 5 minute period of blending at a moderate speed is typical for many samples. A special device for aiding formation of a homogenous sample has been described (49). The device, consisting of a handle and shaped aluminum sheet, fits inside a blender jar and serves to gently push bulky samples into the cutting blades during the blending operation. A liquid-nitrogen cooled freeze grinder for biological materials containing labile pesticides has also been devised (50).

Blending with a solvent followed by filtering or centrifuging is particularly efficient for most vegetable samples. The water in the sample may give

Figure 8-C. Waring Aseptic Dispersall Model AS-1 (Shown on 702-CR Base)



rise to emulsions with nonpolar solvents, and this can often be avoided by use of a drying agent such as anhydrous Na_2SO_4 or 2-propanol together with, or before, the solvent. Meat samples containing too much connective tissue for a blender to deal with effectively should be first comminuted by a grinder. Simple heating of minced sample in a beaker on a steam bath with solvent can be effective, possibly after grinding the sample with Na_2SO_4 and sharp sand to help break down some connective tissue. More volatile pesticides (e.g., lindane) might be lost in this way.

A comparative study of the efficiencies in the extraction of carbofuran from radishes was made using three blenders, a Polytron ultrasonic homogenizer, a Lourdes blender, and a Waring blender. The least efficient blender extracted 90% as much as the most efficient, and all three were considered useful for accurate pesticide analysis (51).

In some cases, more exhaustive extraction of residues from difficult samples can be obtained by Soxhlet extraction for periods up to 12 hours or longer with a solvent such as methanol-chloroform (1:1 v/v) (52). Soxhlet thimbles may require exhaustive extraction prior to use so they do not contribute interferences to the analysis (53). Preliminary steps such as drying, grinding, or chopping normally precede Soxhlet extraction, but care must be exercised since some pesticides have been shown to be unstable in the presence of homogenized samples (54). Even Soxhlet extraction may not give complete extraction in all cases, and only studies with samples to which radioactive tracers have been applied can indicate the absolute extraction efficiency in any particular case. The usual evaluation procedure of spiking a sample with pesticide and looking for quantitative extraction is less reliable than the radiotracer method because the spiked chemical will not be naturally incorporated in the same matrix as would the tracer. Radiotracers are not always available or feasible to use, however. The most important factor in preparation of a valid spiked sample to accurately indicate recovery of endogenous compound may be the solvent in which the spike is dissolved. In one study of the extraction of mirex from fish muscle, recovery varied from 41 to 89% with a common extraction procedure but different spiking solvents (2).

Water samples (100-500 ml) are generally extracted by shaking with an appropriate solvent (3 x 100 ml) in a separatory funnel (55). Soils are extracted by a variety of methods such as shaking, soaking, blending, Soxhlet or Goldfisch extraction, or refluxing. Two 15 minute extractions in an ultrasonic generator were found comparable to a 24 hour Soxhlet extraction for removal of s-triazine herbicides from fortified soils (56), and a 30 second extraction technique using a Brinkmann Polytron ultrasonic generator gave better recoveries of several chlorinated insecticides from soil than did 8 hours of Soxhlet extraction (57).

An apparatus that simultaneously Soxhlet extracts pesticides and concentrates the resulting extract has been designed (58). Advantages of this cyclic extraction-evaporation system are that distillation of solvents prior to extraction can often be omitted, and excess solvent is re-utilized for extraction.

The most efficient solvent and parameters for extraction of pesticides from water can be determined using the p-values originally suggested by Beroza and co-workers for use in residue confirmation (Subsection 10F in Section 10). The p-value is the fraction of total pesticide that is distributed into the nonpolar phase of an equivolume immiscible pair of solvents. This approach was used to study the extraction of OP pesticides from water (59), and the best solvents were benzene, ethyl acetate, or diethyl ether for diazinon and diazoxon at pH 7.4, ethyl acetate for malathion at pH 6, and diethyl ether or ethyl acetate for fenthion (Baytex) at pH 3.4. p-Values can also be used to theoretically select water-to-solvent ratios and the optimum number of extractions for maximum recovery of a pesticide in water (60). As a practical example (61), diethyl ether or ethyl acetate was found best for extraction of 2,4-D acid and esters and benzene for 2,4,5-T acid and esters. A 99% recovery of 2,4-D from one liter of aqueous solution was obtained by a two stage serial extraction with 200 ml and 50 ml of ethyl acetate under conditions predicted by p-values.

8M CONTROL OF METHODOLOGY FOR CONCENTRATION OF SAMPLE SOLUTIONS AND FRACTIONAL COLUMN ELUATES

The concentration of cleaned-up sample in the injection or spotting solution is one important factor that determines if sufficient residue is available for detection by GC, LC, or TLC. The analyst must determine this and concentrate final solutions according to the least sensitive pesticide in the method's scope.

Purified extracts or eluate solutions containing even somewhat volatile compounds are concentrated with minimum losses to a volume of ca 5-10 ml using a Kuderna-Danish evaporative concentrator flask fitted on top with a 3-ball Snyder reflux column and a collection tube on the bottom (Figure 8-D).

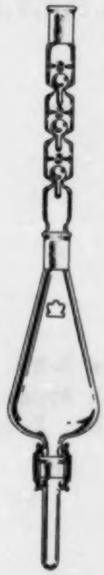


Figure 8-D. Kuderna-Danish Evaporative Concentrator, Kontes Glass Co. No. K-570000.

The tube is heated in steam water bath in a hood. The apparatus should be mounted or held so the lower rounded flask surface is bathed in steam. Flasks, which range in size from 125-1000 ml, should be initially charged with 40-60% of their nominal volume, and the column should be pre-wet with ca 1 ml of solvent before beginning concentration to prevent possible initial small loss of pesticides. Refluxing is continued until the final concentrate is collected in the lower tube. Boiling chips are required for smooth operation of the K-D evaporator, and carborundum, checked for absence of contamination, is recommended in preference to porcelain, vanadium, or glass chips. A Snyder column modified by putting a distillation trap below the Vigreaux bubble condensing system increased the degree and consistency of recovery of nanogram amounts of HCH isomers upon Kuderna-Danish evaporation (62).

For concentration from 5 ml to smaller volumes (as low as 50-100 µl), the concentrate is cooled, the collection tube is removed from the K-D flask, and a fresh chip is added. A micro-Snyder reflux column (Figure 8-E) is fitted directly to top of the tube, and evaporation is begun by holding the bottom of the tube in a steam or hot water bath. Evaporation is continued, with care to avoid bumping, to slightly below the desired volume. The tube is withdrawn from the water when boiling agitation becomes too vigorous; immersion and withdrawal are alternated based on observation of boil agitation. The apparatus is cooled 3-5 minutes, and condensate is allowed to drain down into the tube before the column is removed. The sides of the tube and column joint are rinsed with solvent to avoid hang-up of pesticides on upper glass surfaces. A 1-2 ml syringe is useful for performing this rinse. Finally, further fresh solvent is added to dilute up to the desired volume, if necessary.

A special rack that simultaneously agitates and evaporates solutions in six concentrator tubes fitted with micro-Snyder columns in a time equal to a single tube is described in the EPA PAM, Section 5,A,3,a.

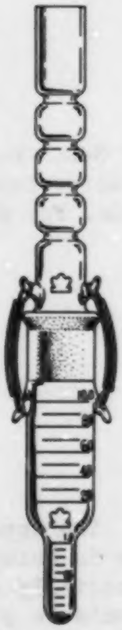


Figure 8-E. Semi-Micro Kuderna Danish Apparatus, Kontes Glass Co., No. K-569250.

Extracts containing fats, oils, or plant extractives, or purified extracts to which "keeper solution" has been added, can be evaporated on a rotating vacuum type evaporator with the water bath at, or just slightly above, room temperature (Figure 8-F). A double-reservoir rotoevaporation vessel facilitating collection, concentration, and final volume calibration of column eluates and eliminating a number of manual transfer steps has been designed (63).

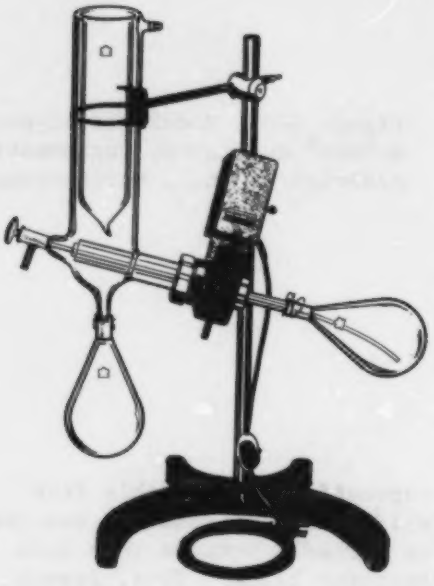


Figure 8-F. Rotary Evaporator, Kontes Glass Co., No. K-570160

Extracts contained in a beaker or a centrifuge tube immersed in a water bath at 40°C can be evaporated under a stream of nitrogen adjusted to cause gentle depression on the surface of the solution. The nitrogen should be passed through well maintained scrubber tubes to remove contaminants that could cause pesticide degradation. Warming a tube by holding it in the hand is a useful, gentle evaporation aid during nitrogen blow-down. Figure 8-G shows the Organomation Associates, Inc., N-Evap apparatus that is widely used for evaporation by nitrogen blow-down.

An evaporation assembly combining an evaporative concentrator tube, a Kuderna-Danish flask, and a rotary vacuum evaporator (Figure 8-F) is shown in Section 10,A of the EPA PAM, Figure 1. The concentrator tube is not immersed in a high temperature water bath as usual, but rather in a 35°C water bath to minimize degradation of heat labile pesticides. This apparatus confines the concentrated extract to one container, thereby eliminating the need for transfer. One hundred ml of methylene chloride can be reduced to 5 ml in ca 20 minutes with a vacuum of 125 mm of mercury.

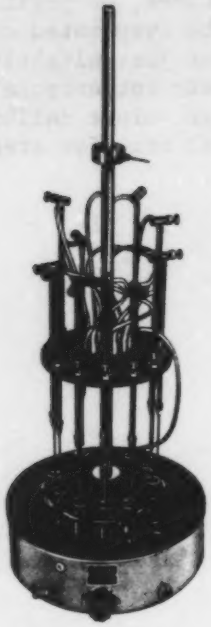


Figure 8-G. Model 111 12-position N-Evap^R apparatus, Organomation Associates, Inc., Northborough, MA.

Another multitube apparatus for nitrogen evaporation is available from Kontes Glass Co. (64). Concentration is rapid until the solution reaches 0.5-1.0 ml, at which point evaporation slows markedly because this last volume is below the heating zone of the evaporator block. Thus, losses of pesticides from inadvertent evaporation to dryness (65) are avoided, and a minimum of analyst attention is required (Fig. 8-H).

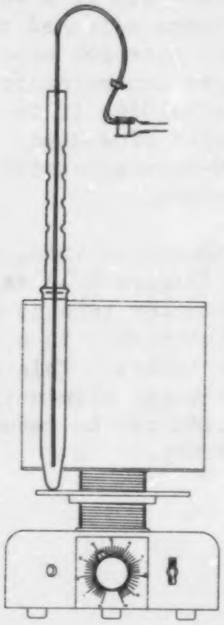


Figure 8-H. Ebullator, Kontes Glass Co.

A distillation column is fixed on top of the tube holding the sample, and small bore stainless steel needlestock tubing is fitted through the column down into the tip of the tube to direct a stream of micro bubbles of nitrogen through the solution to initiate and maintain ebullation. Recoveries of seven chlorinated pesticides after concentration for 2 hours in this apparatus were greater than 94% with both hexane and benzene solvents.

It is important to avoid pesticide loss or decomposition during evaporation steps. Numerous reports have been made (e.g., 65, 66) of severe pesticide loss during concentration steps, even in the presence of sample coextractives. There was no correlation between the amount of coextractives and evaporative losses, but apparently the nature of the coextractives may be important. In most situations, organochlorine and organophosphorus pesticides can be concentrated to small volumes without loss by the K-D evaporative procedures described at the beginning of this subsection. Some recoveries from 100- and 1000-fold concentrations carried out in K-D assemblies are shown in the following table. The recoveries are quite acceptable when concentrating to 1 ml, but when concentrating to 100 µl without a keeper, recoveries become marginal. Using a keeper, such as a paraffin oil, helps retain the compounds and greatly reduces losses. However, the keeper may interfere with some analysis, especially by flame ionization detection or mass spectrometry.

TABLE 8-1

Losses of pesticides on evaporation in Kuderna-Danish concentrators (67)

Pesticide	Original amount in 100 ml hexane (µg)	% Recovery on concentration to *			
		10 ml	1 ml	0.1 ml	0.1 ml with "keeper"
Diazinon	40	102 (2.4)	85 (4.4)	71 (1.8)	83 (1.8)
Aldrin	1.0	103 (2.8)	85 (4.0)	69 (1.2)	81 (0.6)
Malathion	40	85 (2.4)	91 (5.2)	77 (3.0)	88 (0.6)
Parathion	10	93 (4.4)	84 (4.0)	70 (1.2)	82 (2.4)
Dieldrin	2.5	103 (5.6)	92 (4.0)	78 (0.6)	90 (1.2)
p,p'-DDT	5.0	96 (9.2)	91 (5.6)	78 (3.0)	90 (2.4)

* Averages of six determinations for each pesticide. Standard deviations given in parentheses. Gentle stream of nitrogen used to assist concentration below 10 ml.

Evaporation to dryness should never occur. If the complete removal of a particular solvent is required, solvent exchange can be carried out so that the sample never gets to dryness. For example, hexane can be completely removed by boiling-down to a low volume and adding small volumes of acetone as evaporation continues until all hexane is eliminated.

The use of air for concentration of an extract should best be avoided. Satisfactory recoveries are obtainable when the residue levels are relatively high, but significant losses have been documented of even the more stable pesticides at low concentration levels (65).

A commercial tube heater that avoids evaporation to dryness with micro K-D apparatus was originally described by Beroza and Bowman (68) (Figure 8-I). Six extended-tip K-D concentrator tubes are accommodated, and simultaneous evaporation to less than 1 ml can be carried out without attention.

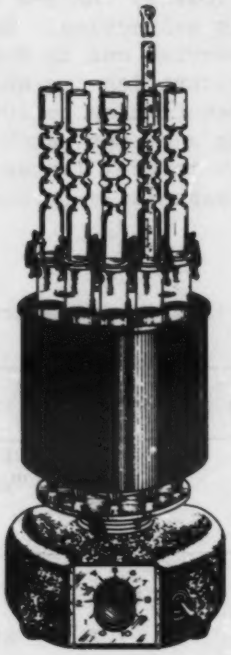


Figure 8-I. Tube Heater, Kontes Glass Co., K-720000

Other reports of pesticide loss include dieldrin and DDT when an extract was evaporated in the presence of light (69), mirex upon evaporation of aqueous solutions (70), and carbamate pesticides when evaporated in a K-D apparatus (71). In the latter case, rotary vacuum evaporation (Figure 8-F) at 50-55°C with addition of a keeper solution was recommended. Many carbamates can be successfully evaporated under a nitrogen stream without loss after adding a keeper. A satisfactory general purpose keeper is 5 drops of 1% paraffin oil in hexane. Solutions containing the herbicide Balan (benefin) cannot be evaporated in a current of air without loss of pesticide, whereas rotary evaporation at a temperature of 50°C or less is successful. All evaporation and concentration steps should be checked with spiked samples if any question of pesticide loss should arise.

The importance of clean glassware in all parts of a pesticide analysis has been stressed several times earlier in this Manual. The special importance of clean glassware to be used for concentration of solutions to small volumes cannot be overemphasized.

The final solution to be used for the determinative step must be composed of a solvent appropriate for the particular analytical procedure. Choice of a volatile solvent for partition and column cleanup procedures is advantageous because evaporation to an appropriate volume can be carried out quickly enough to be practical. If a different solvent is required for the final sample solution, solvent exchange can be carried out by taking up the nearly dry residue in the new solvent after evaporation. Solvents for GC and LC are restricted by the selectivity of the detector, while for TLC almost any volatile solvent is useful for the solution to be spotted. Chlorinated solvents cannot be present in the injected solution when an EC or the Cl modes of the MC or electrolytic detectors are to be used. Acetonitrile has an adverse effect on the response of the EC detector, while aromatic and halogenated compounds and acetonitrile increase the response of the thermionic detector. The most volatile solvent possible should be used to shorten the venting period and minimize loss of early eluting pesticides for those detectors that require solvent venting (e.g., FPD and CCD). A solvent free of UV absorption is required for the detection by the ultraviolet LC monitor.

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Section 9

MULTIRESIDUE EXTRACTION AND ISOLATION PROCEDURES FOR PESTICIDES AND METABOLITES AND RELATED COMPOUNDS

This section presents brief descriptions and quality control aspects of widely used multiresidue analytical procedures for different sample substrates. A few methods for important individual residues are also included. Many of the problem areas are treated in a general manner elsewhere in this Manual, but they are high-lighted again here in relation to the specific methods. References are given in each case to sources of detailed methodology. Control of procedures for collection of samples is covered in Subsections 8A-8K in Section 8 and for sample extraction and extract concentration in Subsections 8L and 8M in Section 8.

CHLORINATED PESTICIDES

9A TISSUE, FAT, AND FOOD ANALYSIS BY THE MILLS, ONLEY, GAITHER PROCEDURE

a. Analysis of Tissue and Fat

The modified Mills, Onley, Gaither method described in Section 5,A,(1),(a) of the EPA Pesticide Analytical Manual has been determined by a number of interlaboratory collaborative studies to yield very acceptable precision and accuracy for the analysis of a number of chlorinated pesticides and metabolites in human or animal fatty tissues. However, many polar OP and carbamate pesticides are not recovered. (This method involves dry maceration of a 5 gram sample with sand and anhydrous Na_2SO_4, isolation of fat by repeated extraction with petroleum ether, extraction of residues into acetonitrile, and then partitioning back into petroleum ether after adding 2% NaCl, drying by elution through a column of Na_2SO_4, concentration of the eluate, cleanup on a Florisil column, and EC-GC after reconcentration of column eluates.) If necessary, further cleanup of the 15% ether-petroleum ether Florisil eluate is carried out on a MgO-Celite column. Pooled blood serum can be analyzed by the MOG Florisil procedure after extraction with a hexane-acetonitrile solvent system [EPA PAM, Section 5,A,(3),(a),VIII].

(1) Some analysts, with hope of saving time, have combined 6% and 15% ethyl ether-petroleum ether Florisil column fractions and have then attempted gas chromatography on the mixture. With some luck this approach might prove successful, but there is a good chance that it could lead to erroneous conclusions. For example, in one documented instance, an analyst reported the presence of aldrin in a human fat sample. Other collaborators on the sample analysis found the same peak in the 15% eluate, making its identification as aldrin impossible since this compound elutes wholly in the 6% fraction. By combining the fractions, the analyst inadvertently neglected the use of selective adsorption as a valuable identification tool.

(2) The polarity of the ethyl ether-petroleum ether eluting solutions exerts a profound effect on the elution pattern of several pesticidal compounds. The amount of ethanol, a relatively polar solvent, in the ethyl ether is a critical factor as illustrated in Figure 4-A in Section 4. As indicated in this figure, with no ethanol, dieldrin would be expected to yield only 87% recovery in Fraction II with the balance being retained on the column. If twice the proper amount of ethanol is present, approximately 7% should elute in Fraction I, giving a 93% recovery in Fraction II. If 2% ethanol is present and all the dieldrin still does not elute in Fraction II, the presence of moisture in the system may be the cause. An excess of moisture may result in all or most of the dieldrin eluting in the 6% fraction.

(3) The activity characteristics of Florisil may vary somewhat from lot to lot. Each lot, when received at a laboratory, should be carefully evaluated to be certain the compound elution characteristics are satisfactory.

(4) Storage and holding temperature of Florisil are critical. The oven used for holding this (and other adsorbents) should be confined exclusively to this usage and not used as an all-purpose drying oven. Florisil will readily pick up air-borne contaminants that may result in spurious chromatographic peaks. If the oven temperature varies more than ± 1°C, considerable influence may be observed in the retention characteristics. The recommended activation temperature is 130°C.

(5) Anhydrous Na_2SO_4 used to top the Florisil column, even AR grade, frequently contains sufficient impurities to result in spurious peaks in the blank eluates. Because of the prevalence of this situation, it is good practice to Soxhlet extract all lots of this salt before use.

(6) The presence of peroxides in ethyl ether can result in extremely low recoveries of organophosphorus compounds and also poses a serious safety hazard. Methods have been set forth for the removal of peroxides from ether but have not proven wholly satisfactory. The purity of petroleum ether is also critical and may exert a profound effect on the recovery of certain of the organophosphorus compounds.

(7) Glassware must be meticulously cleaned to remove electron capturing contaminants. Reagent blanks must be run with each set of samples.

(8) Most chlorinated pesticides should be recovered in the range of 85-100%. HCB is an exception because of an unfavorable partition ratio in the acetonitrile-petroleum ether solvent system. An aldrin spike can be added to the minced fat at the start of the procedure if this pesticide is known to be absent. Recovery of this spike should not be less than 70%.

If improper Florisil fractionation occurs during an analysis, the following points should be considered: Florisil that is too retentive could result from (a) improper activation temperature, (b) improper percentage of ethyl ether in petroleum ether, and ethyl ether that does not contain the required 2% ethanol (read the label on the container carefully). Florisil that appears insufficiently retentive might result from (a) or (b) above, or from residual amounts of a polar solvent in the sample or standard being placed on the column. Likely possibilities are acetonitrile from the sample partition cleanup step (if drying steps are not performed properly) or incomplete removal of benzene (or other solvent more polar than hexane) from a standard solution placed on the column. Other sources of Florisil problems are undoubtedly possible. See also Subsection 9M for further comments on pesticide elution from Florisil.

b. Analysis of Fatty and Nonfatty Foods Using Florisil Cleanup

The Mills, Onley, Gaither column method for determining nonionic chlorinated pesticides in fatty foods is similar to that outlined in Subsection 7Aa and is described in detail in Sections 211, 231, and 232 of the FDA PAM. Eluants are 6, 15, and 50% ethyl ether in petroleum ether. The method for nonfatty foods (FDA PAM, Sections 212 and 232) involves extraction of pesticides with acetonitrile or water-acetonitrile and partition into petroleum ether prior to Florisil column chromatography and EC-GC. The FDA PAM lists pesticides recovered through these procedures [results for some 200 pesticides and other chemicals are given in Table 201-A and over 300 compounds have been tested (1)], samples to which they are applicable, and supplemental cleanup procedures for the Florisil column fractions. This AOAC multiresidue method is currently official for 26 OCl and OP pesticides and PCBs in various groupings of 42 nonfatty and 4 fatty foods (1). The problem areas are the same as those given in Subsections 9Aa and 9M. The elution pattern of more than 150 pesticides from the U.S. FDA Florisil column eluted with 6, 15, 20, 30, 50, and 65% diethyl ether in petroleum ether is tabulated in Section 7.2(b) of the Canadian PAM. Free fatty acids in high quantity are not sufficiently removed by this FDA/AOAC procedure to prevent interference with pesticide determination using electron capture, KCl thermionic, and Hall electrolytic conductivity detectors (2). A potassium permanganate/dilute sulfuric acid oxidation procedure was developed to supplement Florisil chromatography for cleanup of chlorinated pesticide residues in vegetable extracts. Twelve chlorinated pesticides were completely recovered, and only aldrin was lost via decomposition (3).

Thirteen chlorinated pesticides were determined in milk by GC after Florisil column cleanup. Of the several systems tested, extraction of milk with 20 ml hexane plus 5 ml acetonitrile plus 1 ml ethanol produced the highest pesticide recoveries and lowest fat extraction (4).

In order to obtain more efficient cleanup of extracts of fatty foods and recovery of additional pesticides of higher polarity (e.g., organophosphates), a new elution system consisting of three different mixtures of methylene chloride, hexane, and acetonitrile was devised as replacement for the traditional diethyl ether-petroleum ether eluants. These eluant mixtures are methylene chloride-hexane (20:80 v/v); methylene chloride-acetonitrile-hexane (50:0.35:49.65 v/v); and methylene chloride-acetonitrile-hexane (50:1.5:48.5 v/v). At least 50 pesticides and related chemicals have been recovered, in groupings different from the mixed ether systems, with these new solvents (5). Table 201-A of the FDA PAM also includes data on the elution characteristics of compounds using the methylene chloride/hexane/acetonitrile system (FDA PAM, Section 252). A silver nitrate-coated Florisil column has provided cleanup of fatty and vegetable sample extracts and fractionation of chlorinated pesticides and phthalate esters prior to their simultaneous analysis by gas chromatography (6).

Malathion and some other organophosphorus pesticides require 50% diethyl ether-petroleum ether for elution from Florisil. This elution, which must be preceded by elution with the 6% and 15% eluants, has been found occasionally to be inconsistent. OP pesticides can be lost through degradation on the Florisil column and during subsequent evaporations, or when water dilution of the acetonitrile extract for residue transfer to petroleum ether is carried out. Recoveries are tested by carrying known amounts of pesticides through the procedure in the absence of crop substrate. Only 23 of 70 OP pesticides and metabolites tested through the MOG procedure were recovered, and not all recoveries were complete. The AOAC has validated the procedure only for carbophenothion, diazinon, ethion, malathion, methyl and ethyl parathion, and ronnel in 18 fruit and vegetable crops (7-9).

Beckman and Garber (10) recommended the solvent series benzene, diethyl ether-benzene (1:2 v/v), acetone, and methanol for elution of Florisil columns. The elution pattern and recovery of 65 OP pesticides were studied, but sample extracts were not tested. This system was later found to be applicable to the determination of methyl and ethyl parathion, malathion, malaoxon, and paraoxon residues in apples and lettuce, although "all-Florisil" columns were not generally recommended as the best choice for cleanup of OP pesticides (11).

A novel use of Florisil was the development of a partition column consisting of acetonitrile on Florisil for the separation of some pesticides from fish, beef, and butter fat (12). The technique was useful for cleanup of pesticides having favorable p-values (Section 10F) in a hexane-acetonitrile system, which included dimethoate, temephos, methyl parathion, fenitrothion, crufomate, malathion, and parathion.

A multiresidue method for organochlorine, organophosphorus, dinitrophenyl, and carbamate pesticides in applies and other high-water crops was devised using Florisil for cleanup (13). Carbamates were eluted from one column with toluene-acetone (19:1 v/v) and acetylated with trifluoroacetamide for EC-GC determination. Organochlorine and organophosphorus compounds were eluted from a separate column with toluene-acetone (49:1 v/v) and determined by GC. Dinitrophenyl compounds were then eluted from this column with 95% ethanol, cleaned-up by solvent partitioning, methylated, and determined by EC-GC. Most recoveries were greater than 75%, even for polar compounds.

9B HCB AND MIREX IN ADIPOSE TISSUE

Section 5,A,1,(b) of the EPA PAM describes the determination of hexachlorobenzene (HCB) and mirex in fatty tissue with confirmation of HCB by formation of bis-isopropoxytetrachlorobenzene. The sample is dissolved in hexane and applied directly to a Florisil column. The HCB and mirex residues are eluted with hexane and determined by direct EC-GC of the concentrated eluate. HCB is then reacted with 2-propanol, and the BITB derivative is chromatographed to provide confirmation of HCB. Mirex does not survive this reaction. Other common pesticides, some of which are altered by the reaction, are all separated from the HCB derivative on the OV-17/OV-210 column used (14).

9C HUMAN OR ANIMAL TISSUE AND HUMAN MILK ANALYSIS BY THE FLORISIL MICROMETHOD

If the size of the available tissue sample is so small as to make the macro MOG method unsuitable, a micromethod is described in Section 5,A,(2) of the EPA PAM requiring as little as 200-500 mg of sample. The sample is extracted with acetonitrile, pesticides are partitioned into hexane, fractionated on a 1.6 gram Florisil column (eluate I: 12 ml of hexane plus 12 ml of 1% methanol in hexane; eluate II: 12 ml of 1% methanol in hexane), and concentrated fractions determined by EC-GC. Several pesticides, including α-BHC, lindane, diazinon, DDD, and toxaphene, split between fractions. Florisil columns must be conditioned at 130°C at least overnight before using. Precautions concerning use of Florisil are similar to those outlined in Subsection 9Aa. Virtues of the micromethod include a low background level and savings in the volume of solvent required.

Miniaturization of Florisil column cleanup has been reported in several papers (15, 16). One procedure has been successfully studied by several laboratories (17).

9D HUMAN BLOOD OR SERUM

A 2 ml aliquot of serum is extracted with 6 ml hexane for 2 hours on a slow speed rotary mixer. After concentration, the hexane layer is analyzed by EC-GC [EPA PAM, Section 5,A,(3),(a)]. The procedure involves no cleanup, but, if carefully handled, it is capable of yielding recoveries of chlorinated pesticides comparable to that obtained from a full MOG cleanup technique (see Tables 2-4 to 2-9 in Section 2). Since all pesticides will be present in one extract, a GC column must be chosen that will separate the expected pesticides. Certain serum samples will yield a very late eluting extraneous peak (probably a phthalate) that is sometimes large enough to distort a following chromatogram if time is not allowed for its elution from the column. Blood samples should never be stored in containers with polyethylene or rubber caps. Hexane was proven superior to hexane-formic acid for extraction of dieldrin, lindane, and DDT from serum (18). Microcoulometric GC determination after sulfuric acid extraction was successfully applied to 24 organochlorine pesticides in blood at 1 ppb levels with no cleanup (19).

Blood samples are not always analyzed without cleanup steps. Monitoring of fur seal blood for OCl pesticides and PCBs required chromatography of the hexane extract on a 2.3 gram Florisil column prior to EC-GC (20). Another monitoring study of pesticides in human blood was carried out by hexane extraction of acidified samples followed by cleanup on a 1 gram Florisil column and EC-GC (21).

Hexane-acetone (9:1 v/v) was a better extractant for DDT and BHC isomers in human blood than was pure hexane. Maximum recoveries occurred when serum was treated with formic acid before extraction. Ease of extractability decreased in the order: γ-BHC > α-BHC > β-BHC > p,p'-DDE > p,p'-DDT > o,p'-DDT (22).

9E PENTACHLOROPHENOL (PCP) IN BLOOD AND URINE

Acidified blood is extracted with benzene on a Roto-Rack for 2 hours followed by methylation of PCP and determination by EC-GC [EPA PAM, Section 5,A,(3),(b)]. Urine is acidified, and hydrolysis carried out for one hour to free conjugated PCP. PCP and phenol metabolites of PCP and HCB are extracted with benzene, methylated with diazomethane, the methylated phenols are cleaned up and fractionated on an acid alumina column, and determination is carried out by EC-GC [EPA PAM, Section 5,A,(4),(a)]. The following comments pertain to these methods:

a. The alkylating reagent diazomethane is a hazardous chemical and must be handled with extreme caution.*

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*/ Diazomethane and related alkylating reagents (e.g., diazoethane, diazopentane) have been widely used in pesticide residue analysis and are cited in several procedures in this Manual and the EPA PAM. These compounds and their precursors are toxic and carcinogenic and are irritating to the skin. Solutions have been known to explode inexplicably. It is recommended that safer substitutes be found for these reagents whenever possible, for example BF$_3$-methanol for methylation of acid herbicides and acetic anhydride for acetylation of pentachlorophenol (Section 9Ac). Substitution of one reagent for another, however, can require a large amount of effort to check the validity of the procedure with the new reagent. If diazolkane reagents must be used to reproduce established analytical procedures, take care to keep from direct contact with the skin. Wear disposable vinyl gloves and safety goggles, and avoid breathing of vapors. Work behind a safety shield in an efficient hood or inside a radiological glove box. Do not prepare or store reagents in ground glass stoppered or etched glassware. Avoid strong light.

b. A 1.5% OV-17/1.95% QF-1 column is not recommended since the relative retention values for 2,4-D methyl ester and PCP methyl esters are identical and these pesticides would not be differentiated.

c. All reagents including distilled water must be pre-extracted with hexane to remove interfering materials. Reagent blanks should be carried through the entire procedures with each set of samples and standards.

d. Glassware should be washed with dilute NaOH followed by deionized water and acetone.

e. Contact between wooden or paper materials and glassware should not be permitted as some of these materials have been found to contain significant levels of PCP.

f. Other ether derivatives (e.g., ethyl, propyl, amyl, etc.) can be prepared and characterized for confirmation of PCP identity.

g. Fortified samples should be analyzed along with each series of actual samples to verify adequate recovery of PCP and the other phenols of interest. Because of the ubiquity of PCP, the "blank" used for fortification must be analyzed, and a correction must be made for the amount of PCP found.

h. A reagent blank consisting of 5 ml of pre-extracted distilled water should also be carried through the entire procedures along with samples.

i. Confirmation of PCP is based on chemical ionization mass spectrometry or extraction $\underline{p}$-values.

In the methods described above (23, 24), phenols were chromatographed on conventional GC columns after derivatization to a more easily chromatographed compound. The derivatization step exposes the analyst to a toxic derivatizing reagent and increases the possibilities of error. It has been demonstrated that support coated polyester columns are suitable for determining free chlorinated phenols in urine at subnanogram levels without the need for derivatization (25).

A method for monitoring PCP in fish and other environmental samples with a ± 2% accuracy and precision has been described (26). PCP was extracted from fish tissue and converted to pentachloroanisole (PCA) by means of alkylation in the presence of potassium carbonate as a condensing agent. After adding pentachlorophenetole as internal standard, determination was carried out by electron impact mass fragmentography monitoring 280 m/e for PCA and 294 m/e for the internal standard.

9F BIS(p-CHLOROPHENYL)ACETIC ACID (p,p'-DDA) IN HUMAN URINE

The excretion level of this metabolite is a sensitive indicator of exposure to p,p'-DDT. Urine is extracted three times with an equal volume of 2% acetic acid in hexane, the combined extracts are evaporated to remove residual water or acetic acid, DDA is converted to its methyl ester by reaction with BF_3-methanol reagent, and the ester is extracted with hexane and determined by GC with microcoulometric or EC detection [EPA PAM, Section 5,A,(4),(b)].

Microcolumn Florisil cleanup (Subsection 9C) is required when the poorly selective EC detector is used. DDA should elute completely in Fraction II. Concentration and injection volumes depend upon the sensitivity of the detector employed. A column of 5% OV-210 at 175-180°C will separate DDA from p,p'-DDE (which usually is also present in high exposure donors), whereas 4% SE-30/6% QF-1 or 1.5% OV-17/1.95% QF-1 columns at 200°C will not resolve these compounds.

A very similar procedure involving diazomethane methylation, no cleanup, propyl ester internal standard, a 1% QF-1 column at 190°C, and a ^{63}Ni pulsed EC detector has been reported. The calibration curve was linear up to 1.5 μg DDA per liter, the coefficient of variation was ca 8%, the absolute detection limit was 0.05 ng, and 20-30 samples could be run per day (27).

9G 2,4-D AND 2,4,5-T IN URINE

A method is described in the EPA PAM, Section 5,A,(4),(c), for determining these herbicides and their degradation products 2,4-dichlorophenol and 2,4,5-trichlorophenol in human and animal urine. Phenolic conjugates are hydrolyzed in acid, and free phenols and acids are extracted with benzene and ethylated with diazoethane. Cleanup and fractionation of derivatives is carried out on a silica gel column (1 gram, containing 1.5% water), and determination of concentrated eluates by EC-GC on a 4% SE-30/6% OV-210 column.

Deactivated silica gel (Subsection 4Ad in Section 4) columns should be prepared just prior to use. Because of the differences in temperature and humidity from one laboratory to another, silica gel elution parameters should be established by each analyst under local conditions. The percentage water added for deactivation should be increased if the compounds of interest elute in a later fraction than that indicated in the detailed procedure, or, the percentage of benzene in the benzene-hexane eluant can be increased. Early elution would be remedied by less deactivation or less polar solvents. Spiked control urine, rather than standard compounds, should be used to determine the elution pattern. See the footnote on page 6 concerning the hazards associated with the ethylating reagent. Alkylated standards are stable for one month if stored in a freezer (-18°C) when not in use.

A multiresidue scheme for phenol metabolites and including 2,4-D, 2,4,5-T, and silvex is discussed in Subsection 9R. A method for monitoring 2,4-D in the urine of pesticide spray operators at 0.1 ppm involved cleanup on XAD-2 resin, quantitation by GC of the methyl ester, and confirmation by trans-butylation to the n-butyl ester. Recovery was 94 ± 6% for five fortified samples (28).

9H KEPONE IN HUMAN BLOOD FOR ENVIRONMENTAL SAMPLES

The determination of Kepone in human blood, air, river water, bottom sediments, and fish is described in the EPA PAM, Section 5,A,(5),(a). This is based on the research of Moseman *et al.* (29), Hodgson *et al.* (30), and Harless *et al.* (31). Samples are extracted, and the extracts are cleaned up by chromatography on a micro Florisil column, base partitioning, or gel permeation chromatography. Kepone is determined by EC-GC with multiple columns. Confirmation is by chemical conversion to mirex followed by further cleanup prior to EC-GC (32); detection with a Hall conductivity detector in the Cl-mode; or chemical ionization mass spectrometry (31).

It is mandatory to use 1-2% methanol in benzene for all sample and standard solutions injected for EC-GC to obtain the maximum reproducible response. Sufficient control and spiked reference materials should be utilized to ensure the validity of analytical results for all sample types. Elution patterns for the Florisil columns should be carefully established by each analyst by eluting standard Kepone under local laboratory conditions. Analytical standards should be validated by cross-reference analysis of difrerent preparations of analytical grade Kepone with agreement within ± 5% of the established purity.

The analysis of field-collected avian tissues and eggs for Kepone residues has been reported (33). Samples were extracted with benzene-isopropanol (2:1 v/v) and extracts cleaned up with fuming H_2SO_4-concentrated H_2SO_4 (1:1 v/v). Separation of Kepone from OCl pesticides and PCBs was obtained on a 10 gram 130°C-activated Florisil column eluted with 100 ml of benzene-acetone (95:5 v/v) followed by 200 ml of benzene-methanol (90:10 v/v); the second eluate contained the Kepone. Determination was by EC-GC on a 4% SE-30/6% QF-1 column and confirmation by GC-MS. Recoveries averaged 86% at 1 ppm. Procedures for determination of Kepone in serum, plasma, urine and fat have been reported. After addition of H_2SO_4, samples were extracted with hexane-acetone (17:3 v/v), extracts were evaporated, and the residue dissolved in benzene-methanol (99:1 v/v). The extraction was modified for feces and bile. Programmed temperature GC with pulsed EC detection on a 4% SE-30/6% QF-1 column provided linear calibration curves for 10 pg-100 ng of Kepone (5 ppb-50 ppm/gram sample) (34). Determination of Kepone in eels (35); blue fish or shrimp (36); finfish, shellfish, and crustaceans (37); water and sediment (38); and soil and mullet (39) using gas chromatography have also been reported in the literature.

9I GEL PERMEATION CHROMATOGRAPHY

a. Gel Permeation Chromatographic Cleanup of Adipose Tissue

(1) Theory

Gel permeation chromatography (GPC) is a form of liquid chromatography by which compounds are separated according to molecular size. It is particularly useful in separating very large molecules such as lipids and cholesterol found in adipose tissue samples from the smaller molecules of pesticides, PCBs, etc. The method is as effective as the MOG procedure for cleanup in pesticide residue analyses (40) and has the added advantages that removal of fat is more complete and recoveries of pesticides are nearly quantitative. Hence, it is the ideal choice for GC-MS analyses, where maximum detectability of pesticides is needed and minute quantities of lipid materials can cause serious interferences.

Porous polymer beads (e.g., BioBeads SX-3) are used as gel particles and organic solvents (e.g., toluene, ethyl acetate, or cyclohexane) are used for the mobile phase. The elution process is very simple (isocratic only); the same solvent system is used for column preparation, elution, and washing. Macromolecules cannot permeate the porous gel and are rapidly eluted or "dumped" from the column. Molecules that can enter the pores of the beads are temporarily retained to greater or lesser extents depending on their molecular volumes. Hence, large-volume pesticides such as mirex elute first (in this case, following shortly after cholesterol), while small-volume pesticides such as HCB elute last. Since molecular volume rather than molecular weight dictates the order of elution, all equatorial β-BHC elutes after the other BHC isomers.

(2) Equipment

The gel permeation chromatograph is an AutoPrep Model 1001 (Analytical Biochemistry Laboratories, Inc., Columbia, MO), equipped with a 2.5 cm id x 60 cm glass column (Chromaflex^R R-422350/6025, Kontes, Vineland, NJ, or equivalent) packed with 200- to 400-mesh BioBeads SX-3 (BioRad Laboratories, Richmond, CA).

(3) Column Preparation and Operation

(a) Prepare a slurry of ca 60 g of BioBeads SX-3 in pesticide quality (or equivalent) toluene-ethyl acetate (1:3 v/v). This will be sufficient to pack a column about 25 cm long.

(b) Add small volumes of resin and solvent to the column. Each addition of resin must be in contact with enough solvent to swell the resin before the next addition.

(c) After the resin is transferred to the column, compress the gel to approximately 25 cm, allowing solvent to flow out of the column exit.

(d) Add only ethyl acetate-toluene (3:1 v/v) to the solvent reservoir. Addition of other solvents to the system via sample introduction will change the gel swelling ratio and must be kept to a minimum (i.e., < 5% v/v of aliquot injected).

(e) Install the column and start the pump. The pump operating pressure should be 5-7 psi (not to exceed 10 psi).

(f) Adjust the pumping rate to approximately 5 ml/minute with the pump vernier control valve.

(g) Set the timer to collect for 20 minutes and check the actual pumping rate.

(h) The GPC elution pattern of the pesticides of interest should be established for standards before introduction of biological samples into the gel permeation chromatograph.

(4) Procedure for GPC Cleanup

(a) Start up the GPC instrument and elute the column with ethyl acetate-toluene (3:1 v/v) until it is purged of entrained air.

(b) Introduce ≤1 g of sample in 5-8 ml of eluting solvent into the sample introduction valve. Rotate to the next sample loop and introduce the next sample. After the last sample is loaded rinse the sample valve with clean solvent. Up to 500 mg of lipid can be injected onto the GPC column.

(c) Set "dump" and "collect" cycles to previously established rates for elution of the pesticides of interest (usually 100-125 ml and 125-225 ml, respectively).

(d) For multiple sample cleanup, an appropriate "wash" cycle of ca 50 ml ethl acetate-toluene (3:1 v/v) should be included after every sample.

b. Elution Data

Since 5,B of the EPA PAM describes the elution profiles of some 100 pesticides of all classes (organochlorines, PCBs, organophosphates, carbamates, thioureas, etc.) from the AutoPrep 1001 gel permeation chromatograph using a 50 g, 30 x 2.5 cm column of BioBeads SX-3 gel and cyclohexane-methylene chloride (85:15 v/v) and ethyl acetate-toluene (3:1 v/v) eluants.

c. GPC Combined with Alumina Column Cleanup

Section 5,B of the EPA PAM describes the use of cyclohexane-methylene chloride (85:15 v/v)-BioBeads SX-3 GPC cleanup coupled with a deactivated mini-alumina column for improved purification of pesticide extracts from fat samples. In many cases, GPC fractions require no further cleanup prior to determination of residues by GC.

d. Application of GPC

The original GPC system consisting of BioBeads SX-2 crosslinked polystyrene gel and cyclohexane was designed by Stalling et al. (41) for removal of lipids from extracts of samples such as fish before EC-GC determination of commonly occurring pesticide and PCB residues. This excellent method was later improved considerably by the use of BioBeads SX-3 and ethyl acetate-toluene. A broad range of OCl and OP pesticides can be recovered in good yields from fats and oils (42, Subsection b above). An evaluation of the GPC system (43) with different sample types indicated that ca 98% of the fat or oil content of the extract is generally eluted prior to the pesticide fraction and that this cleanup may be superior to that achieved by acetonitrile partition and Florisil adsorption. However, although recoveries were higher by GPC than by Florisil adsorption, precision was poorer with the former method. Analyses can be automated since an important feature of GPC is that the same column can be used repeatedly over long periods without significant change in elution volumes or recoveries.

With the GPC procedure described in Subsection a above, organochlorine pesticides have been determined and confirmed in human tissue and milk (EPA PAM Section 12,A). Samples are extracted and cleaned up by a modified Mills, Onley, Gaither procedure. After further cleanup of Florisil fractions by GPC, determination is carried out by GC on a Carbowax 20M column with a Hall electrolytic conductivity detector.

Recoveries ranging from 88-106% were reported for disulfoton, diazinon, methyl parathion, malathion, parathion, dichlorvos, and fensulfothion in an evaluation study of the automated gel permeation chromatographic cleanup techniques using BioBeads SX-3 gel and an ethyl acetate-toluene (3:1 v/v) elution solvent (42). A solvent composed of cyclohexane-methylene chloride (85:15 v/v) with BioBeads SX-3 provided adequate cleanup for EC-GC (no liquid partitioning) of 9 OP pesticides and 2 metabolites and 16 nonionic OCl pesticides in vegetable oils at 0.05-1.0 ppm. Vegetables, fruits, and crops were analyzed for 26 organophosphorus pesticides and metabolites at 0.05-0.10 ppm levels using automated GPC for cleanup followed by FPD-GC. Recoveries of 7 compounds from 12 sample types were in the range 83-103%, and 8 compounds could be determined simultaneously (44). Carbamate and organophosphorus compounds in several plant crops were recovered at levels between 82 and 104% by automated GPC (45).

Gel chromatography on Sephadex LH-20 has also been reported (46, 47) for cleanup of organochlorines and organophosphates prior to GC, but this approach is now of minimal importance in residue analysis.

9J DETERMINATION OF CHLOROPHENOXY HERBICIDES IN FATTY AND NONFATTY FOODS

Section 221 of the FDA PAM covers the analysis of chlorophenoxy acid herbicides in fatty foods. The pesticides are extracted from the foods and are partitioned into alkaline solution, and the extract is washed with organic solvents and is acidified. Chlorophenoxy acids are re-extracted into chloroform and methylated after evaporation of the chloroform. Florisil chromatography of the methylated extracts provides cleanup and separates the methyl ether of PCP from the chlorophenoxy acid methyl esters. PCP methyl ether is determined by electron capture GC, and the phenoxy acid esters by microcoulometric or electron capture GC, depending upon the sample type. The recovery of seven compounds from fatty samples has been verified. Methylated acids are easily lost during evaporation steps, especially from standard solutions. Solvents should be evaporated very carefully in a gentle stream of nitrogen. Traces of soap that are not rinsed away can give interference peaks, even with the selective microcoulometric detector.

Chlorophenoxy acids are extracted from nonfatty foods with acetonitrile or acetonitrile-water. After evaporation of the acetonitrile, the aqueous extract is made alkaline, the alkaline solution is washed with organic solvents, and it is then acidified. The acids are extracted into chloroform and then methylated, and the methylated extract is cleaned up and fractionated as above (FDA PAM, Section 222). The recovery of residues of seven compounds in nonfatty foods has been verified. An alternative alumina and Florisil column cleanup prior to EC-GC for the ester fraction from vegetable samples is given in the Canadian PAM, Section 7.3.

A bibliography of extraction and analytical procedures suitable for monitoring phenoxy herbicides in plants, animals, and the environment has been published (48).

9K CARBON-CELLULOSE COLUMN CLEANUP

Section 7.1 of the Canadian PAM describes a method for cleanup of residues of organochlorine and organophosphorus insecticides, herbicides, and fungicides in foods following acetonitrile extraction (blending) and hexane partition. Sequential elution with three solvents (1.5% acetonitrile in hexane, chloroform, and benzene) separates the pesticides into three fractions that are suitable for EC-GC and TLC determination. Some 40 pesticides have been quantitatively recovered from a variety of foods with this system. A carbon-cellulose (4:10 w/w) minicolumn eluted with ethyl acetate has been used for cleanup and separation of phorate and metabolite residues in crops determined by FPD-GC (49).

The Canadian PAM specifies that carbon (e.g., Darco G60) is prepared by heating for 12 hours at 300°C and then extracting for two one hour periods with hexane on a mechanical shaker. See also Section 4Af in this Manual. Cellulose (e.g., Solka Floc BW40) is extracted twice with acetone in a similar manner without prior heating.

9L CLEANUP ON SILICA GEL

Silicar CC-4 silica gel (50) has been widely used for cleanup and fractionation of OCl insecticides in various monitoring programs (51). For example, in a study of duck wing contamination (52), a 15 g column was eluted with 60 ml of petroleum ether (HCB, mirex recovered), 350 ml of petroleum ether (PCBs, some DDE), and 150 ml of methylene chloride-hexane-acetonitrile (80:19:1 v/v) (remainder of DDE, TDE, DDT, and other OCl compounds). The same elution sequence was used to determine OCl residues in herons (53). A modified sequence with four eluants, used to assess contamination of Bald Eagles, allowed collection of dieldrin and endrin in a discrete fraction: 80 ml petroleum ether (HCB and mirex); 320 ml petroleum ether (PCBs, PBBs, DDE); 275 ml hexane-methylene chloride (85:15 v/v) (OCl compounds, except endrin and dieldrin); 200 ml methylene chloride-hexane-acetonitrile (80:19:1 v/v) (endrin and dieldrin) (54).

9M CLEANUP ON DEACTIVATED FLORISIL AND SILICA GEL (see also Section 9G)

The method of Osadchuk et al. is described in the Canadian PAM, Section 7.2. Deactivated Florisil is prepared as outlined in Subsection 4Ac in Section 4 of this Manual. The elution behavior of over 50 pesticides on Florisil deactivated with 2% water has been determined for use after extraction and partition cleanup of residues. A 30 cm x 2.5 cm id column containing 15 cm of adsorbent is eluted with 300 ml portions of the appropriate eluting mixture(s) ranging from pure hexane to 5-30% methylene chloride in hexane to 5-30% ethyl acetate in hexane (Table 9-1). If the analyst wishes to screen a sample extract for a larger number of pesticides in one or two GC injections, the less polar eluants may be by-passed and only the more polar used. However, some sample types may be inadequately cleaned-up by this procedure or mutually interfering residues may occur in the same fraction.

The following factors affect the success of this Florisil procedure:

a. Pesticides containing a mercaptan function are oxidized on the Florisil column. For example, phorate, captan, carbophenothion, chlorobenside, disulfoton, and demeton have losses ranging from 20-100%. The oxidation proceeds to the sulfoxide and then to the sulfone. Therefore, non-detection of such pesticides does not guarantee they were not originally present in the sample. The degree of oxidation by Florisil increases with a lower extent of water deactivation (greater adsorbent activity) or a greater time of contact with the column and may also be affected by the pH of the particular Florisil used.

b. Oxygen analogs of organophosphorus pesticides are strongly adsorbed on Florisil and cannot be completely eluted even with very polar solvents.

c. The 2% deactivated Florisil column can tolerate up to one gram of fat or oil (30% methylene chloride in hexane or less polar eluants) without extraneous EC-GC response.

d. Up to two grams of fat or oil can be applied directly to the column and eluted with 10% methylene chloride in hexane to recover BHC isomers, the DDT group, PCBs, and HCB.

Table 9-1

ORDER OF ELUTION OF PESTICIDES FROM FLORISIL PARTIALLY DEACTIVATED WITH 2% WATER USING 300 ml VOLUME OF ELUENTS

(From the Canadian PAM)

PESTICIDES	1	% CH_2Cl_2 in Hexane						% EtOAc in Hexane				Percent Recoveries
		Hexane	5%	10%	15%	20%	30%	5%	10%	20%	30%	
Aroclor 1254	PCB	+										>95
Chlordane		+										>95
Toxaphene		+										>95
Strobane		+										>95
trans-Chlordane		+										>95
Chlordene		+										>95
Aldrin		+										>95
Hexachlorobenzene		+										>95
Heptachlor		+										>95
p,p'-DDE		+										>95
o,p'-DDT		+										>95
Mirex		+										>95
Isobenzan		+[2]										>95
p,p'-DDT		S(90%)	+									>95
α-BHC		S(45%)	+									>95
Perthane		S(10%)	S(85%)	+								>95
p,p'-DDD			+									>95
Chlorbenside	M		+[2]									~60
γ-BHC			S(80%)	+								>95
PCNB			S(80%)	+								>95
TCNB			S(45%)	+[2]								>95
β-BHC			S(10%)	+								>95
δ-BHC					+							>95
Dicofol					+							>95
Ronnel	OP				S(65%)	+						>95
Hepachlor epoxide					S(60%)	+						>95
Dichlofenthion	OP					+						>95
Phorate	OP,M					+						~ 20
Carbophenothion	OP,M					S(25%)	+					~80
Endosulfan I						S(10%)	+					>95
Dieldrin							+					>95
Chlorpyrifos	OP						+					>95
Endrin							●					>95
Methoxychlor							●					>95
Parathion	OP							+				>95
Ethion[2]	OP							+				>95
2,4-D methyl ester								+				>95
2,4,5-T methyl ester								+				>95
Anilazine								+				>95
Ovex								+				>95
Fenitrothion	OP							S(90%)	+			>95
Tetradifon								S(90%)	+			>95
Diazinon	OP							S(90%)	+			>95
Chlorothalonil								S(70%)	+			>95
Methyl Parathion[3]	OP							S(50%)	+			>95
Sulphenone								S(15%)	+			>95
Dioxathion	OP								+			>95
Malathion	OP								●			>95
Atrazine[4]									+			>90
Simazine[4]									+			>90
Endosulfan II									S(75%)	+		>95
Captan	M									+		~80
Phosmet	OP									+		>95
DCPA										+		>90
Azinphosmethyl	OP										+[2]	>95

Note: A 30% CH_2Cl_2 fraction was eluted prior to all ethyl acetate fractions. All others were single elutions.

+ - mostly elutes in first 250 ml
● - large amount in 250-300 ml fraction
S - some (as percent)

Footnotes:
1. OP = organophosphorus; M = mercaptan; PCB = polychlorinated biphenyl
2. Higher recoveries are obtained by elution with more polar eluents
3. Remaining methyl parathion elutes in another 50 ml of 5% EtOAc
4. Detected by alkali flame detector

The approach in d above has been used to determine HCB and mirex in fish and butterfat by elution with acetonitrile from a column composed of the fat or oil distributed on unactivated Florisil. This procedure has been collaboratively studied (55) and adopted by the AOAC as official final action (56). Care must be taken in analyses for HCB not to use plastic wash bottles, since this compound was found as a contaminant in 30 of 34 such bottles tested (57).

A one-step Florisil column cleanup described by Langlois et al. (58) has been widely used to isolate organochlorines and PCBs. It is similar to the method just described but employs deactivated Florisil. Activated Florisil is equilibrated with 5% water, and 1 g of fat from fish or other extracts is thoroughly mixed with 25 g of this Florisil. The adsorbent is placed on top of a second 25 g portion of conditioned Florisil in a 25 mm id column. The column is eluted with 300 ml of hexane-methylene chloride (4:1 v/v) (59).

Silica gel deactivated with 30% water has been used to isolate organochlorines from lipids (60). A micro column of this silica gel eluted with petroleum ether has been shown (61) to yield especially pure eluates. Small columns of precisely deactivated silicic acid (3 g, 3.3% water) were found to separate p,p'-DDT, cis- and trans-chlordane, p,p'-DDE, and PCBs from the majority of toxaphene components. This fractionation greatly simplified the analysis of the pesticides (62).

9N LOW TEMPERATURE PRECIPITATION

This procedure (Canadian PAM, Section 7.4) is used to separate fats, oils, and water from acetone-benzene-acid extracts of biological samples by precipitation at -78°C. The special low temperature cleanup apparatus is described in detail (Canadian PAM, Section 14.5). Many apolar and polar residues and metabolites (e.g., DDT, 2,4-D acid and ester, parathion, and paraoxon) are retained in the acetone supernate and can be determined by EC-GC. Forty pesticides have been quantitatively (80+ percent) recovered from a variety of plant and animal products at levels greater than 0.05 ppm. Freeze-out has been recently employed for the removal of lipids prior to Florisil chromatography and EC-GC in the determination of methoxychlor residues in microsamples of animal tissues and water at 10 ppb and 1 ppb levels, respectively (15), and for cleanup of human milk samples for HCB and other chlorinated pesticides by EC-GC (63).

90 CLEANUP ON ALUMINA

Hexane extracts of animal tissues are cleaned-up and prefractionated on narrow bore columns dry-packed with partially deactivated alumina and silica gel by the method of Holden and Marsden (64). The initial alumina column eluted with hexane provides removal of lipids, while the second column affords pre-GC separation of residues plus further cleanup. Table 9-2 shows the elution order of chlorinated insecticides with hexane and 10% diethyl ether-hexane eluants. Alumina is activated at 800°C and silica gel at 150°C before deactivation with 5% (w/w) water. Interferences contributed by columns in the Holden-Marsden method have been removed by methylene chloride treatment of the columns. Basic alumina was recommended for easier control of activity and faster pesticide elution (65).

Another alumina-silica column scheme (66) was devised for separation of 17 OCl residues in 4 eluates, each containing pesticides separable on a 4% SE-30/6% OV-210 GC column. Microcolumns deactivated with 3-4% water were used.

Table 9-2

ORDER OF ELUTION OF ORGANOCHLORINES FROM DEACTIVATED SILICA GEL ACCORDING TO THE METHOD OF HOLDEN AND MARSDEN (64)

Eluted in order by hexane	Eluted in order by 10% diethyl ether in hexane
Hexachlorobenzene	Endrin
Aldrin	Chlordane
PCBs	p,p'-DCBP
p,p'-DDE	Toxaphene
Heptachlor	p,p'-TDE
p,p'-MDE (DDMU)	Telodrin
o,p'-DDT	Heptachlor epoxide
p,p'-DDT	α-BHC
	Perthane
	β-BHC
	Kelthane
	γ-BHC
	Dieldrin
	Methoxychlor

Organochlorine insecticide residues in fatty foodstuffs were determined (67) by using a cleanup technique based on a single 22 g column of activity-4 basic or neutral alumina. Concentrated hexane extracts of samples, containing 0.4-0.5 grams of fat, were transferred to the column, and pesticides were eluted with 150 ml of hexane prior to determination by EC-GC.

Recoveries of 15 insecticides from vegetable oil samples spiked at levels of 5-250 μg/kg were between 70-124%. Routine determinations were carried out for cyclodienes, BHC isomers, and HCB at the 5-10 μg/kg level and DDT-type compounds at the 20-30 μg/kg level. Results of collaborative studies were reported. If PCBs were present, the column was eluted with 10 ml and then 150 ml of hexane. The first fraction contained all the PCBs and all or most of any residues of aldrin, heptachlor, HCB, p,p'-DDE and o,p'-DDT. The second fraction contained all the BHC isomers, heptachlor epoxide, dieldrin, endrin, p,p'-DDD, methoxychlor, and Endosulfan A. Compounds splitting between fractions included methoxychlor, toxaphene, perthane, chlordane, and strobane. Further collaborative study (68) of the method found it satisfactory for determining residues of hexachlorobenzene and β-HCH in butterfat and mutton fat; α-HCH, γ-HCH, p,p'-DDT, and p,p'-DDE in chicken fat; β-HCH, dieldrin, hexachlorobenzene, and TDE in pork fat; DDT isomers in eggs; and other OCl insecticides in these and other samples of animal origin.

A microcolumn of 2.0 g of Woelm basic alumina deactivated with 11% water has been used for cleanup of water extracts and fractionation of residues. Petroleum ether (5 ml) eluted HCB, α- and γ-BHC, heptachlor epoxide (10%), p,p'-DDE, o,p'-DDT, TDE, p,p'-DDT, telodrin, isodrin, aldrin, and heptachlor. Subsequent elution with 10 ml of petroleum ether-ethyl ether (80:20 v/v) recovered β-BHC, heptachlor epoxide (90%), dieldrin, and endrin (69).

In a comparative study (70), basic alumina was found to retain lipids better than Florisil, which in turn held more than silicic acid. It was also found that deactivation and elution with less polar solvents gave a superior separation of organochlorine pesticides from lipids than activated adsorbents and more polar eluants. Saponification with ethanolic NaOH followed by alumina column chromatography provided efficient removal of lipids prior to GC determination of several OCl insecticides (DDT was converted to DDE) (71). A procedure for evaluation of the fat capacity of an aluminum column has been described (68).

9P MISCELLANEOUS MULTIRESIDUE CLEANUP PROCEDURES

Other multiresidue procedures include the following: The method of de Faubert Maunder (72) employs partition with dimethylformamide (DMF) to diminish the amount of fat carried over with the pesticides from fatty samples. A hexane extract of the sample is extracted three times with hexane-saturated DMF; the combined DMF phases are washed with a DMF-saturated hexane and then shaken with a large volume of 2% Na_2SO_4 solution. On standing, a hexane layer containing chlorinated pesticides forms on top of the solution, this layer is separated, and residues are cleaned-up on an alumina column and determined by EC-GC. Like the AOAC-MOG procedure (Section 9A), this method does not give good recoveries of hexachlorobenzene from fatty samples.

Wood (73) proposed a rapid method for small samples using dimethyl sulfoxide (DMSO). This is a good solvent for chlorinated pesticides that dissolves only low amounts of oil or fat. The fatty sample is mixed with Celite

(1:15 w/w) and packed into a small column, and the pesticides are eluted with DMSO. The eluate is adsorbed directly onto the top of a larger Florisil column and the residues then eluted with hexane from the Florisil. The method does not seem to be widely used.

The de Faubert Maunder and Wood methods have been compared with the standard FDA-AOAC Florisil procedure for analysis of chlorinated pesticides in a variety of foodstuffs (74). No gross general differences were found in results, but one method might be advantageous for a particular sample type.

A rapid DMSO-petroleum ether partitioning cleanup method employing test tubes and syringes in place of separatory funnels was found to recover 60 OCl, OP, and carbamate pesticides at levels > 50%. Losses were found to be consistent, so the use of correction factors was proposed. Crops containing 0.1-10 ppm levels were tested for analysis by GC (EC and FPD detectors), TLC, and HPLC (75).

A reuseable, macroporous silica gel column provided fractionation and 88-105% recoveries of 0.1-1 ppm levels of different classes of pesticides when eluted with a series of solvents of increasing polarity (76).

Thin layer chromatography (TLC) on 1-5 mm layers can provide cleanup if a minimal amount of fatty material is present in the extract. Sample is applied as a streak and developed along with standard marker compounds on the same plate to allow location of the pesticide zones. These bands are removed by scraping and are extracted to recover the separated pesticides. Modified layers have been devised with capability for increased sample loading, e.g., multiband or wedge-layer chromatoplates (77). With the latter, cleanup and determination can be combined on the same layer without intervening elution.

The use of ion exchange resins for cleanup of ionic pesticides has been reviewed (78). For example, acidic residues such as chlorophenols and phenoxy acids in extracts of organic tissues, soil, and water will bind under alkaline conditions to a strong base anion exchange resin. After washing out impurities, the residues can be eluted from the resin column by an acidic eluant and determined by EC-GC after appropriate derivatization reactions (79).

The results of international cooperative studies of OCl pesticide, PCB, and Hg residues in wildlife have been reported (80). The analytical methods were based on extraction, cleanup, and GC determination, but no two laboratories used exactly the same procedure. Nonetheless, there was reasonable agreement among laboratories in analysis of test samples, the coefficient of variation for different chlorinated compounds ranging from 10-17%. Collaborative testing of a multiresidue method for chlorinated hydrocarbon and other fumigant residues among 8 foreign laboratories was successfully completed, and results were reported (81).

ORGANOPHOSPHORUS PESTICIDES

9Q DETERMINATION OF METABOLITES OR HYDROLYSIS PRODUCTS IN HUMAN URINE, BLOOD, AND OTHER TISSUES.

The determination of intact organophosphorus pesticides in tissue or blood from suspected poisoning victims is described in Section 6,A,(1) of the EPA PAM (82). However, in cases of low exposure or in high exposure cases after several hours, the probability of detecting parent compounds is greatly reduced because of rapid metabolism (83). In most instances, the determination of alkyl phosphate metabolites in urine provides a measure of the extent of human exposure to the parent OP pesticide. Section 6,A,(2),(a) of the EPA PAM and reference (84) contain a sensitive and selective analytical procedure for alkyl phosphate and phosphonate metabolites (hydrolysis products) of important pesticides.

OP metabolites in urine are extracted quantitatively with an anion-exchange resin after addition of acetone in a 10:1 ratio to precipitate some interfering compounds. The compounds are eluted from the resin, derivatized with diazopentane (see footnote on page 6 for precautions when using this reagent), and the derivatives determined by FPD (P-mode)-GC. If very low levels of alkyl phosphate metabolites are present, further cleanup on a 2.4 gram silica gel column deactivated with 20% water is carried out. Confirmation is by FPD-GC using both the P and S detector modes (recall that the S-mode is 5 to 10 times less sensitive). Analysis can be made at the 0.1 ppm level, so that the excretion of alkyl phosphates in urine can be detected at pesticide levels much lower than those that result in cholinesterase inhibition. The general class of organophosphate pesticide (but not the exact compound) involved in the exposure may be deduced by characterizing the metabolite(s) excreted. These analytical methods have been applied to the analysis of the urine of rats exposed to a group of aromatic and aliphatic OP and phosphonate pesticides (85).

Because of the complexity of this method, routine analyses should be validated by simultaneous analysis of spiked SPRM's. As outlined in Section 3, one SPRM is analyzed along with each unknown if only occasional analyses are performed, or the ratio of SPRM to routine analyses is at least 10% when larger numbers are involved. Because of the possible instability of urine samples spiked with alkyl phosphates, large samples of SPRM should not be prepared ahead of time for periodic analyses. A method for preparation of individual SPRM as needed is detailed in Section 6,A,(2),(a),XI of the EPA PAM. However, it has been shown (86) that dialkyl phosphate metabolites do not break down or disappear in urine samples frozen for up 20 weeks prior to analysis.

Underivatized compounds may accumulate on the GC column after periods of extended use. Injection of 1 µl of diazopentane solution should be made every two weeks to react with these compounds. If peaks appear following this injection, the column should be reconditioned (Subsection 4I in Section 4). Further confirmation of any particular metabolite can be accomplished by preparing its hexyl derivative.

Reproducibility of this method is not as good as is desirable for a reliable, routine analytical method. This can be seen in Table 3 in EPA PAM Section 6,A,2,(a), where recovery variabilities of 15% or greater are reported for six analyses at the two highest spiking levels. In the study of freezer storage of alkyl phosphate metabolites described above (86), the method was found to give both low and highly variable recoveries. Because of the unreliable quantitation obtained, the method currently described in Section 6,A,2,(a) of the EPA PAM should be considered only semi-quantitative.

In addition to alkyl phosphates, significant amounts of the corresponding mono- and dicarboxylic acids are found in the urine of humans exposed to malathion. A silica gel cleanup FPD-GC method for determining these acids as a measure of exposure to malathion has been devised (87). Urine is extracted, the extract is alkylated, and derivatized carboxylic acids are cleaned up according to a previously published (88) alkyl phosphate method. Additional cleanup by solvent partitioning with ether and silica gel chromatography [elution with benzene followed by ethyl acetate-benzene (10:90 v/v), collected as one fraction] is also employed. Derivatized MCA and DCA are determined on a 4% SE-30/6% QF-1 column at 200°C.

A reportedly simple and rapid method for quantitation of the metabolites of malathion and other OP pesticides has been published (89). The omission of an extraction at low pH and the mild condition of anion-exchange chromatography on QAE-Sephadex prevented degradation of a malathion metabolite that takes place under strongly acid conditions. Disadvantages of the commonly used partition fractionation of malathion and malaoxon metabolites were discussed.

Another new method also employing an ion exchange resin for determination of mono- and diportic alkyl and aryl phosphates, phosphonates, and thio analogs in human urine has been reported to have a detection limit of less than 2 pmole for each of these classes of compounds. The acids were protonated by passing through a hydrogen-form cation exchange resin. Benzyl esters were formed by refluxing the column effluent with 3-benzyl-1-*p*-tolyltriazene in acetone, partitioned into cyclohexane, and determined by GC (5% OV-210 column) with a P-mode FPD. Inorganic *o*-phosphate did not interfere, but could be removed by calcium hydroxide precipitation if desired (90).

Urinary dialkyl phosphate metabolites have also been determined using 1-(4-nitrobenzyl)-3-(4-tolyl)triazene as derivatizing reagent. Urine was lyophilized, dialkyl phosphates were derivatized, and cleanup was carried out by anhydrous nickel sulfate adsorption and silica gel chromatography. GC analysis determined the metabolites at levels as low as 0.01 ppm (91).

9R DETERMINATION OF *p*-NITROPHENOL (PNP) AND OTHER PHENOLS IN URINE

Urinary PNP, the phenolic metabolite of ethyl and methyl parathion, EPN (O-ethyl O-*p*-nitrophenyl (phenylphosphonothioate) nitrofen, etc., can be measured as an indicator of exposure to these organophosphorus pesticides. A small volume of urine is hydrolyzed with HCl to form free PNP, then made alkaline and cleaned-up by extraction with benzene-ether, and finally re-acidified and extracted with benzene-ether to remove PNP. An aliquot of dried extract is analyzed by EC-GC with on-column conversion of PNP to the volatile trimethylsilyl derivative [EPA PAM, Section 6,A,(2),(b)].

A multiresidue analytical procedure for halo- and nitrophenols from a range of biodegradable pesticides (organophosphates, phenoxy acids, organohalides) is also useful for determining exposure to these pesticides (92, 93). A one to five ml sample is treated with a 1/5 volume of concentrated hydrochloric acid and the mixture refluxed at 100°C for one hour. The phenols are extracted with diethyl ether, ethylated by reaction with diazoethane, and the ethyl ethers chromatographed on a silica gel column (2 grams, 2% water deactivation). (See the footnote on page 6 concerning precautions when using diazoalkanes). Elution with various concentrations of benzene in hexane purifies and fractionates the phenolic ethers, which are finally determined by EC-GC.

Ten phenols, including the pesticides pentachlorophenol and DNOC (4,6-dinitro-o-cresol), plus the herbicides 2,4-D, 2,4,5-T, and silvex can be determined by this scheme on one sample. All halogenated phenols are eluted with 20% benzene-hexane, while nitrophenols and phenoxy acids elute in the 60 and 80% fractions. The phenoxy acids are detected intact along with 2,4-dichlorophenol and 2,4,5-trichlorophenol, their potential mammalian metabolites.

A method for the determination of residues of the herbicide DNBP (2-sec-butyl-4,6-dinitrophenol) in feed, blood, urine, feces, and tissues by EC-GC has been devised in the EPA Health Effects Research Laboratory (94). After extraction, the sample is reacted with diazomethane (see footnote on page 6 concerning precautions when using diazoalkane) to produce the methyl ether of DNBP. Cleanup and recovery of the derivative is obtained on acid alumina column eluted with hexane-benzene (40:60 v/v). Average recoveries of greater than 85% were obtained from samples fortified at 0.1-30 ppm levels.

9S SWEEP CO-DISTILLATION

Sweep co-distillation has proven to be a simple time saving cleanup technique that eliminates the need for specialized adsorbents and large volumes of purified solvents (8, 95-100). The technique can be used for OCl and OP residues in fruits and vegetables, or fats and oils. The procedural details are different for the two sample types; however, the cleanup principle is essentially the same. The concentrated sample, in an organic solvent, is injected into a heated tube swept with 600 ml N_2/min. Sample extractives remain in the tube while volatilized components are swept into a simple condensing train. After a 30 minute sweep time, the transfer lines are disconnected and condensed pesticides are rinsed with organic solvent into the sample tube. After volume adjustments, the sample may generally be analyzed by GC without further cleanup. If sensitivity levels in the low part per billion range are desired, an auxillary cleanup is recommended. The combination of sweep co-distillation and the micro Florisil column [EPA PAM Section 5,A,(2)] has proven to be a thorough cleanup for fat samples. A sulfuric acid/Celite column can be adapted as an optional automatic cleanup step (101).

Figure 9-A is a schematic diagram of the apparatus as originally used for cleanup of fruits and vegetables for determination of OP residues. The glass wool-packed tube was placed inside a heated copper tube. A nitrogen sweep of 600 ml/min was used. Two gram aliquots of sample were injected followed by ethyl acetate injections every three minutes. See FDA PAM Section 232.2 for method details.

OCl and OP residues in a variety of edible fats and oils have been determined by a modified version of the sweep co-distillation cleanup system (102, 103). A tube packed with glass wool, sand, and glass beads is operated in a vertical position with the injection port on bottom. The cleanup is effected by the 250° heat and the nitrogen carrier gas distributing the oil upward through one-half to three-fourths of the glass bead packed column with a percolation type action. Pesticides are volatilized and swept into the collector trap. Recent study of sweep co-distillation of fats has shown that follow-up injections of solvent are not necessary. After initial injection of the sample, the equipment may be left unattended for the 30 min sweep operation.

Figure 9-B shows the appearance of a commercial version of the "Sweep Co-distiller" (Kontes Glass Co., Vineland, NJ). This apparatus permits simultaneous cleanup of four samples with a 30 min sweep time. The 30 cm tube allows efficient cleanup for OCl or OP residues in samples of fats, oils, milk, and crops (operated in vertical position at 250°C with 600 ml N_2/min) (104). The tube for fat cleanup may be purchased prepacked, but packing in the laboratory is preferable for consistent tube uniformity. The empty tube may also be prepared for fruit and vegetable cleanup by packing with 15 cm glass wool in the injection end with remaining space filled with glass beads. The oven would be swiveled to a horizontal position for the fruit and vegetable cleanup. Operational parameters for the latter application may be found in the FDA PAM, Section 232.2.

In a preliminary evaluation of the Kontes apparatus by Watts, common organochlorine pesticides were quantitatively recovered from chicken fat, and the fat residue was reduced to less than 1% of the original sample. Similar results have been obtained by Luke with both the Kontes and laboratory-assembled sweep-co-distillation units. An oven temperature of 227-230°C was used, and no solvent injections were made after the sample was applied. Reproducible, quantitative recoveries were obtained for organophosphorus and organochlorine pesticides from beef fat and butterfat (105).

The operating principle of sweep co-distillation has been presented diagrammatically, and recoveries of 36 OP pesticides in 20 substrates (0.03-0.5 ppm) and 30 OCl pesticides in 14 substrates (0.003-0.05 ppm) are tabulated (106).

Figure 9-A. Sweep co-distillation apparatus, schematic diagram.

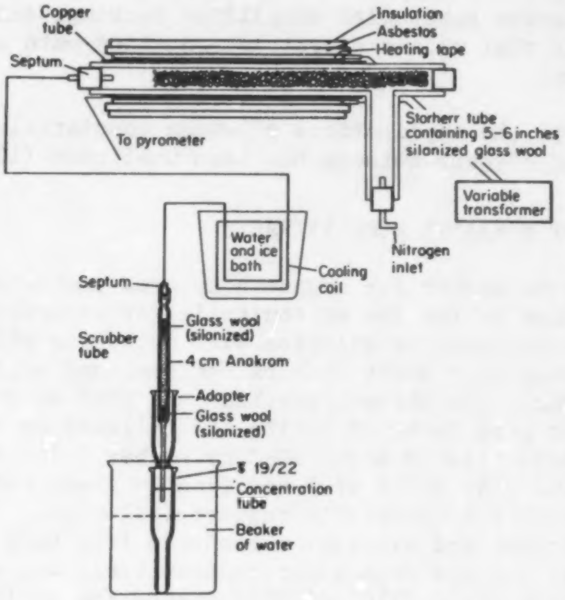
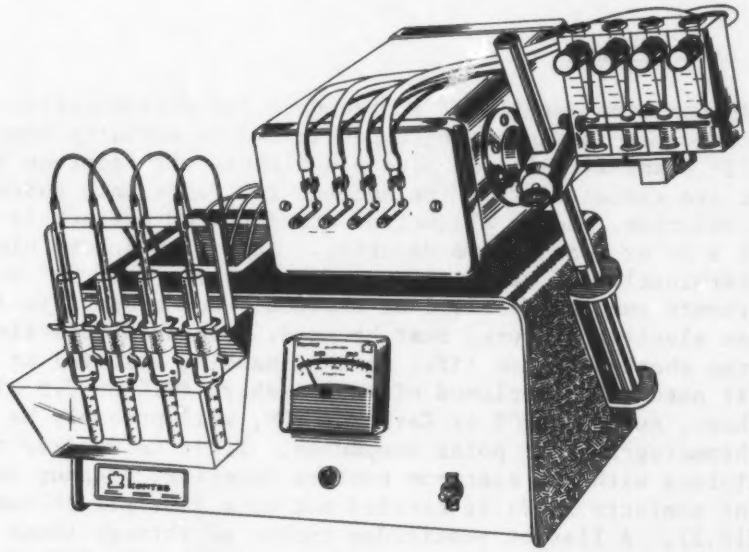

Copper tube
Insulation
Asbestos
Heating tape
Septum
Storherr tube containing 5-6 inches silonized glass wool
To pyrometer
Variable transformer
Water and ice bath
Nitrogen inlet
Cooling coil
Septum
Glass wool (silonized)
Scrubber tube
4 cm Anakrom
Adapter
Glass wool (silonized)
¥ 19/22
Concentration tube
Beaker of water

Figure 9-B. Sweep co-distillation apparatus, (oven positioned for fruit and vegetable cleanup), Kontes Glass Co., K-500750.



Heath and Black (107) recommended the following modifications for faster and more convenient cleanup of organochlorine residues in animal fat: no solvent introduction; 230°C distillation temperature; 600 ml/minute nitrogen flow; 6.7 mm distillation tubes with simplified packing; and incorporation of a U-tube condenser that allows direct introduction onto a Florisil column for secondary cleanup.

A literature review of the applications of sweep co-distillation including a comparison to other cleanup methods has been published (108).

9T CHARCOAL CLEANUP OF NONFATTY FOOD EXTRACTS

A general determinative method for organophosphorus pesticide residues in nonfatty foods is based on the FDA acetonitrile (or water/acetonitrile) extraction procedure followed by dilution with methylene chloride to separate water, cleanup on a short charcoal column, and analysis by GC with a P-selective detector. The chromatographic tube (300 mm x 22 mm id) is packed dry with a one gram layer of Celite 545 followed by 6 grams of adsorbent mixture (acid-treated Norit SG-X or Nuchar C-190 charcoal-hydrated magnesium oxide-Celite 545, 1:2:4 w/w) and finally glass wool topping, and the column is eluted with acetonitrile-benzene (1:1 v/v). The satisfactory recovery of 41 pesticides and alteration products from kale and 9 typical pesticides from other low and high sugar content crops was demonstrated (109). A collaborative study (110) of this method for residues of six OP compounds in apples and green beans verified recoveries between 86 and 125% when either a thermionic or FPD detector was employed. The method is described in the FDA PAM, Section 232.3., and recoveries of 51 pesticides and related chemicals are listed in Table 201-H of the FDA manual. Sections 4Ae and f of this Manual describe procedures for purification of Celite and carbon adsorbents, respectively.

9U ACETONE EXTRACTION

The FDA PAM contains details of a procedure for determination of polar organophosphate and organonitrogen pesticides in nonfatty samples (FDA PAM Sections 232.4 and 242.1). Samples are blended with acetone and filtered, pesticides are extracted from the aqueous filtrates into petroleum ether-methylene chloride, and an aliquot of concentrated extract is determined by GC with a P- or N-selective detector. Lack of a column cleanup step allows determination of many polar compounds that would not be recovered from adsorbents such as Florisil or charcoal, but a specific detector, rather than electron capture, must be used. Repeated injection of impure extracts can shorten column life, so that packing material at the head of column will need to be replaced often. A short (0.6 or 0.9 m) column of a polar phase, such as DEGS or Carbowax 20M, will probably be advantageous for the chromatography of polar compounds. If it is desired to examine some pesticides with the electron capture detector, cleanup of acetone extracts of nonfatty foods is carried out on a Florisil column (FDA PAM Section 212.2). A list of pesticides recovered through these procedures, with and without Florisil cleanup, is given in the FDA PAM, Table 201-I.

9V MISCELLANEOUS MULTIRESIDUE CLEANUP PROCEDURES

Nine extraction procedures were compared for efficiency of removal of six OP pesticides and metabolites from field treated crops. Soxhlet extraction of the finely chopped crops with chloroform-methanol (90:10 v/v) proved most reliable and efficient (111).

Alumina has not proven totally satisfactory for cleanup of OP compounds since recovery of the more polar compounds is not complete (112). Using alumina (activity II to III) and petroleum ether and petroleum ether-acetone (97:3 v/v) as eluants, Renvall and Akerblom (113) eluted only 13 of the 31 OP compounds they tested. However, many residue analyses are based on alumina column cleanup, e.g., the determination of carbophenothion in goose tissues (114) and monocrotophos in tobacco (115) by FPD-GC.

The Abbott _et_ _al_. method (116), involving cleanup by solvent partition without column chromatography, has proven adequate for analyses of seven types of foods for 39 pesticides and metabolites when detection was made with a thermionic detector. Finely chopped sample is mixed with anhydrous sodium sulfate and extracted with acetonitrile. The extract is diluted with a large volume of aqueous sodium sulfate, and the pesticides are extracted into chloroform. The chloroform solution is dried and concentrated for GC. Other determinations without column cleanup have been reported. Methyl parathion, diazinon, malathion, and phorate were determined in plant, animal, water, and soil samples by EC-GC following only hexane extraction and partition with aqueous acetonitrile (117). Azinphosemethyl and dimethoate residues in apple leaves were determined by FPD-GC following ethyl acetate extraction and cleanup by methylene chloride-water and hexane-acetonitrile partitionings (118). A multiresidue analysis of 14 pesticides in natural waters at ppb levels involving extraction and concentration before FPD-GC has been reported (119).

The elution pattern of a series of representative OP pesticides from a column (Kontes, Size 22) containing one gram of Woelm silica gel deactivated with 1.5% water and prewashed with 8 ml hexane before applying the sample mixture is as follows:

Eluant	Pesticides Eluted
7 ml hexane	-----
8 ml 60% benzene-hexane	carbophenothion
8 ml benzene	ethyl parathion
8 ml 8% ethyl acetate-benzene	malathion, diazinon
8 ml 50% ethyl acetate-benzene	paraoxon

Silica gel or silicic acid columns have been used for cleanup of animal, plant, soil, and water extracts prior to GC determination of OP pesticides (120-122) and to separate OP pesticides and metabolites into groups to facilitate their identification by GC (123). A tandem column of silica gel and alumina was used to separate leptophos and its oxygen and 2,5-dichlorophenol analogs prior to determination by FPD-GC (124).

A rapid, simple approach has been developed for approximately the total residues of pesticides such as fenthion, disulfoton, and phorate, which may consist of the parent pesticide and up to five metabolites formed by oxidation of thionophosphate and sulfide groups in each molecule. The insecticides and any metabolites are oxidized to the oxygen analog sulfone with m-chloroperbenzoic acid, followed by removal of the acid on an alumina column and determination of the sulfone by FPD-GC. Quantitative recoveries of parent pesticides and metabolites from corn, milk, grass, and feces have been demonstrated (125). Metasystox-R and its sulfone were determined in plant and animal tissues and water at 10 ppb levels as the sulfone after oxidation by $KMnO_4$(126).

A method for 40 organophosphorus pesticide residues in plant material involved extraction with acetonitrile, partition into methylene chloride, and GC with a P-selective thermionic detector (127). The same authors used this extraction and cleanup procedure for plant material subsequent to oxidation with potassium permanganate to convert organophosphorus pesticides containing thioether groups (e.g., demeton, disulfoton, phorate) to sulfones (128).

A collaborative study by 12 laboratories of the methods of Abbott et al. (116; see above, this Subsection), Watts et al., and Sissions and Telling was conducted for OP pesticides in fruits and vegetables (129). The method of Abbott et al. was found satisfactory for determination of malathion, dichlorvos, dimethoate, omethoate, and parathion in 6 fruit and vegetable crops (>90% average for all pesticides and crops at 0.5-2 mg/kg) and was judged widely applicable to the determination of many other nonpolar and medium-polarity OP pesticides and to a wider range of samples. The method of Watts et al. (130), involving ethyl acetate extraction and cleanup on a column of activated charcoal-magnesium oxide-Celite eluted with ethyl acetate-acetone-toluene (an early version of the procedure described in Section 9T), was found satisfactory for the same pesticides plus azinphos-methyl in 6 crops (>90% average recovery at 0.5-2 mg/kg) and was also judged to be much more widely applicable. The method of Sissions and Telling (131), employing cleanup by batch addition of charcoal followed by hexane and hexane-acetone (98:2 v/v) elution through an activity -5 alumina column was not successful for the more polar pesticides studied. Details and modifications of these methods are discussed in the report of the collaborative study.

The methods of Abbott et al. (116) and Sissons and Telling (112) and the sweep co-distillation method (Section 9S) were compared for determination of different OP pesticide residues in various vegetable crops. There was no significant difference for most pesticide-crop combinations, except that sweep co-distillation tended to give lower results for polar compounds such as omethoate (132).

A tabulation has been made (133) of the validated applicability of five multiresidue analytical methods to the determination of some 50 OP insecticides, acaricides, and nematocides. These procedures were the AOAC (11th ed.) 29.001-29.027 general Florisil cleanup method for OCl and OP pesticides; the AOAC (11th ed.) 29.028-29.033 multiple residue carbon column cleanup method for OP pesticides; the AOAC (11th ed.) 29.034-29.038 single sweep oscillographic polarographic confirmatory method; the Abbott et al. method for total diet studies (116); and an undescribed German procedure (134). In addition, individual determinations of some of the compounds by other special methods were reviewed. It was stated that, in general, the multiresidue methods were not usually suitable for metabolites, requiring separate analysis for the parent and metabolite; each method should be compound validated in the worker's own laboratory; and that differences in results were more likely to arise from sampling problems than from the analytical methods themselves.

The use of a selective detector sometimes allows determination of OP pesticides with no cleanup. For example, a collaborative study of the analysis of wheat for chlorpyrifos methyl, fenitrothion, malathion, methacrifos, and pirimiphos methyl involved only methanol extraction for 40 hours followed by GC of an aliquot using a FPD or alkali flame ionization detector (135).

CARBAMATE PESTICIDES AND METABOLITES AND MISCELLANEOUS HERBICIDES

9W 1-NAPHTHOL IN URINE

Humans exposed to the N-methyl carbamate insecticide carbaryl excrete in urine relatively large quantities of the metabolite 1-naphthol conjugated as either the sulfate or glucuronide. Determination of 1-naphthol is made by subjecting 5 ml of urine to acid hydrolysis under reflux to break conjugates, extracting the 1-naphthol with benzene, and derivatization with chloroacetic anhydride solution. After cleanup on a small silica gel column (1 gram, 1.5% water), the derivative is quantitated by EC-GC against a peak from standard 1-naphthol similarly derivatized. Details are found in Section 7,A of the EPA PAM.

Elution patterns from the silica gel column must be established at the temperature and humidity conditions prevalent in each laboratory. Spiked control urine treated in the same manner as routine samples is used for this purpose. Traces of water can affect the derivatization reaction and must be avoided. Derivatized standards are stable for about 6 months if stored in a refrigerator.

9X ANALYSIS OF AMINE METABOLITES IN URINE

A method for determination of amine metabolites from anilide, urea, and carbamate pesticides was developed in the EPA Research Triangle Park Laboratories (136). Pentafluoropropionic anhydride was the preferred derivatization reagent for the aniline compounds, with cleanup on 1 gram deactivated (3) percent water) silica gel columns. Determination was by EC-GC on a 3% OV-1 column. Recoveries ranged from 85-90% at 1.0 and 0.1 ppm.

9Y OTHER INDIRECT (DERIVATIZATION) METHODS OF ANALYSIS

Numerous derivatization methods have been used for the indirect measurement of residue levels of parent carbamate insecticides in a variety of agricultural crops and other substrates. These have involved derivatization of the amine or phenol moieties of the pesticides after hydrolysis, or, less often, the intact insecticide. These derivative methods include reaction of intact insecticides with bromine, silylating reagents, acetic anhydride, and trifluoroacetic anhydride. Phenols resulting from alkaline hydrolysis of the parent insecticides have been reacted with bromine (with or without simultaneous esterification), silylating reagents, mono- and trichloroacetyl chloride, pentafluorobenzyl bromide, and 1-fluoro-2,4-dinitrobenzene. The latter reagent is used for derivatization of carbamate insecticides in the method for water analysis (Section 9A,C) discussed in this Manual and described in detail in the EPA PAM, Section 10,A.

Amine hydrolysis products of carbamate insecticides have been reacted with 1-fluoro-2,4-dinitrobenzene and 4-bromobenzoyl chloride. These and other reactions have been surveyed in a review article (137) in which pertinent references are given.

GC methods for phenyl substituted urea and carbamate herbicides are usually based on hydrolysis followed by determination of the corresponding aniline. Anilines have been derivatized with halogen, 4-chloro-α,α,α-trifluoro-3,5-dinitrotoluene, 1-fluoro-2,4-dinitrobenzene, and pentafluoropropionic anhydride. These reactions are also reviewed in reference (137).

A fluorogenic labeling derivatization reaction with dansyl chloride has been combined with HPLC for the determination of N-methylcarbamate insecticides in soil and water. No preliminary cleanup was required, and detection limits were 1-10 ng/4 μl injection (138).

Ten triazine herbicides were determined in vegetables at levels of 0.13-0.86 ppm by preparation of heptafluorobutyryl derivatives. Compared to the parent compounds, the products were at least 300 times more sensitive to electron capture detection and 5-10 fold more sensitive to Cl-mode electrolytic conductivity detection (139).

9Z DIRECT METHODS OF ANALYSIS

Determinations of intact, underivatized N-methylcarbamate insecticides are hampered by their decomposition on GC columns under ordinary operating conditions (140). Losses can be minimized by the use of specially prepared, conditioned, and maintained columns. Presilanized supports do not provide sufficient deactivation to prevent degradation of carbamates, so it is necessary to employ in situ silanization both during initial conditioning and thereafter to restore column performance. Examples of direct analyses of crop extracts include a multiresidue method (141) on 5-6% DC-200 after acetonitrile partition and charcoal cleanup as for OP pesticides (109), determination of carbofuran and other carbamates on 20% SE-30 (142), and determination of 0.2-15 ng of carbaryl and 1-naphthol on a short column of 3% SE-30 (143). Highly deactivated GC columns prepared from acid washed Chromosorb W support that is surface modified with Carbowax 20M have also been successfully used for chromatography of intact N-methylcarbamates without degradation on the column. Such columns, which are extremely promising for performing analyses without required derivatization, are described in Sections 4J and 5Lb of this Manual.

Urea and N-arylcarbamate herbicides are, in general, more thermally stable than carbamate insecticides, and are, therefore, more amenable to direct determination by GC. For example, columns of 5% E-301 methyl silicone at 150°C (144), 10% DC-200/15% QF-1 (1:1) at 160°C (145), and 5 and 10% DC-200 (146) have been successfully used, the former for multiresidues of urea herbicides and the latter two for carbamate herbicides in foods. However, decomposition of compounds on these column types has been noted under certain conditions, and determinations are therefore often made via thermally stable derivatives of hydrolysis products or directly on Carbowax 20M-treated columns. As an example of the latter, carbamate insecticides and herbicides have been directly chromatographed on Carbowax 20M modified (Ultra-Bond) supports containing 1-3% of a liquid phase such as OV-17, OV-101, or OV-210. The Hall electrolytic conductivity detector was used, and determinations in soil were demonstrated (147).

s-Triazine herbicide residues were determined in urine by hexane extraction from a sample at pH 12, drying of the extract by passage through a sodium sulfate column, concentration of the extract, and GC using a N-mode Hall conductivity detector (148). Similar N-specific GC methods involving cleanup were used to monitor triazines in European streams (149).

9A,A ANALYSIS OF PLANT AND FOOD MATERIALS

Extraction of urea and carbamate pesticides from plant materials usually involves blending with methylene chloride, acetone, chloroform, acetonitrile, or an alcohol (or these solvents plus anhydrous Na_2SO_4). If the presence of conjugates of hydroxy metabolites is suspected, hydrolysis with an acid during extraction may be included (Section 9A,L).

Cleanup steps include solvent partition and/or liquid column chromatography, the exact nature of which are pesticide- and sample-dependent.

For example, a column of 4:1 MgO-cellulose was used for cleanup of carbamate herbicide residues from a variety of foods (145), while Florisil was employed after acetonitrile-petroleum ether partition for the multiresidue, multiclass determination of carbamate, urea, and amide residues (150). Methods for extraction, cleanup, and GC of carbamates, ureas, and other classes of herbicides (triazines, uracils, phenols) have been reviewed (151-153). A multiresidue method for twelve triazine herbicides in crops, water, and soils involving methanol extraction, alumina column cleanup, and gas chromatography with a Carbowax column and thermionic, microcoulometric, FPD, and electrolytic conductivity detectors has been reported (154). Residues of 15 organonitrogen herbicides and fungicides were screened in foods by acetone extraction, partition and Florisil (2% water) column cleanup, and CCD-GC determination (155). Herbicides of different types were determined in crops at tolerance levels with no column cleanup prior to GC with N- and Cl-mode conductivity detection (156). Total residues of Mesurol and its sulfoxide and sulfone metabolites in plant and animal tissues were determined by oxidation of the extract with $KMnO_4$ to convert all residues to the corresponding sulfone, which was detected at a limit of 0.03 ppm by a S-mode FPD (157).

9A,B AIR ANALYSIS

Section 8,B of the EPA PAM contains details of analytical methods for chlorinated, organophosphorus, and N-methylcarbamate insecticides collected by one of the procedures described in Section 8,A of the EPA PAM or Section 8H of this Manual. The sampling medium is extracted with hexane-diethyl ether (95:5 v/v). Chlorinated pesticides and PCBs are measured by EC-GC after column chromatographic cleanup on alumina. PCBs are separated from technical chlordane and other pesticides by column chromatography on silicic acid deactivated with distilled water. Organophosphorus pesticides are determined by direct injection of an aliquot of extract into a chromatograph equipped with a flame photometric detector. Carbamate pesticides are determined directly by GC using a N-mode Hall detector and a 3% OV-101/Ultra Bond 20M column. As an alternative for carbamates, derivatization is carried out with α-bromo-2,3,4,5,6-pentafluorotoluene. The derivatives are cleaned-up and fractionated on a column containing 1 g of deactivated silica gel and determined by EC-GC. Collection efficiencies of OCl and OP pesticides and PCBs using different samplers and collection media as determined with these analytical procedures are tabulated in Section 8,B of the EPA PAM.

The air analysis method reported earlier in the EPA PAM was a multiclass, multiresidue procedure (158) for residues collected in ethylene glycol, in which prefractionation was carried out on a 1 g column of silica gel deactivated with 20% water. A still earlier method for air analysis (159) included Florisil column chromatography and was the basis of the former EPA National Pesticide Monitoring Program (160). Details of these can be found in earlier editions of the EPA PAM, but use of the current, more widely applicable and tested procedures, described above, is generally recommended.

9A,C WATER ANALYSIS

A broadly applicable multiresidue, multiclass method for the monitoring of water samples for pesticides is presented in Section 10,A of the EPA PAM (161). Recovery studies were conducted on 42 halogenated compounds, 38 OP compounds, and 7 carbamates. Recoveries of >80% were achieved for 58 of the 87 compounds, 60-80% recovery for 13 compounds, and <60% for the remaining 16 compounds (concentration levels 0.09-400 ppb). Pesticides are extracted from water with methylene chloride, and the concentrated extract is chromatographed on a 1 gram deactivated (20% water) silica gel column with four different solvents of increasing polarity to separate the pesticides into groups. OCl compounds are determined by EC-GC, OP compounds by FPD-GC, and carbamates by EC-GC after conversion to 2,4-dinitrophenyl ether derivatives. Low recoveries were in most cases traced to losses during the silica gel chromatography step. Evaporation of solutions by air blowdown should not be used because losses of all three classes of pesticides may occur. Concentrations are carried out under a gentle stream of nitrogen. It is important to apply the concentrated extract to the silica gel column at the exact moment the last of the hexane prewash reaches the top surface of the column. The total 0.5 ml extract plus the 1.0 ml hexane rinse must be transferred to the column without loss to minimize the recovery error. Solvents contained in several eluate fractions from the silica gel column may interfere in the GC and carbamate derivatization steps. It is critical to follow the directions for solvent removal and exchange outlined in Section 10,A of the EPA PAM. Sufficient silica gel should be activated (at 175°C) to provide only a one-week supply, and deactivation should be carried out only on the amount required for a 2 or 3 day period. Longer storage periods may result in a shift of the pesticide elution pattern of the final deactivated columns. Each lot of silica gel should be tested for the proper elution pattern with representative pesticide standards eluting in each fraction. A number of the OP compounds require considerable column pre-conditioning by repetitive injection of high-concentration standards in order to obtain linearity of response and accurate quantitation. Confirmation of pesticide identity should be made by several techniques outlined in Section 10.

Section 10,B of the EPA PAM describes the determination of some free acid herbicides (e.g., MCPA, 2,4-D, 2,4,5-T) in water. The water is adjusted to pH 3 and extracted with methylene chloride. The extract is taken to dryness, pesticides are esterified with 10% BCl_3 in 2-chloroethanol, and the resulting esters are extracted with hexane, concentrated, and determined by EC-GC. If cleanup is required, chromatography on silica gel deactivated with 20% water is employed. This procedure is a further extension of the multiclass, multiresidue procedures described directly above. When preparing the BCl_3-chloroethanol esterification reagent, work in an efficient exhaust hood and wear disposable vinyl gloves because 2-chloroethanol is toxic by dermal contact or when inhaled.

The reagent is stable for at least thirty days if kept stoppered and refrigerated. As usual, spiked reference material containing the same pesticides at comparable concentrations as in the sample (if these are known) should be analyzed in parallel. Other aspects of quality control are as discussed in the preceding paragraph. BCl_3-methanol was also chosen in another study (162) as the best derivatization reagent for the determination of 8 phenylalkanoic acid herbicides in water (0.01-2.5 µg/L); solvent partition and silica gel (5% water deactivated) minicolumn cleanup and EC-GC with an OV-17/QF-1 column were employed.

The 1979 Analytical Methods Manual of the Inland Waters Directorate, Water Quality Branch, Environment Canada, Ottawa, contains detailed methods for the analysis of organochlorinated pesticides and PCBs, organophosphorus pesticides (two procedures), phenoxy acid herbicides (two procedures), pentachlorophenol, and N-methyl carbamates in waters. The method for organochlorines, employing benzene extraction, Florisil column cleanup, and EC-GC, has detection limits ranging from 0.001-0.01 ppb. The first OP procedure determines dimethoate, fenitrothion, and phosphamidon and the second determines 14 other OP pesticides, all at 0.005-0.1 ppb levels by FPD-GC without cleanup. Phenoxy acid herbicides (2,4-D; 2,4,5-T; Silvex) are extracted with chloroform from acidified water and converted to their methyl esters utilizing BF_3-methanol prior to cleanup on a Florisil column and EC-GC determination at 0.01 ppb levels. A second procedure determines 8 phenoxy acid herbicides at 0.01-2.5 µg/L levels by extraction of acidified water with ethyl acetate, back extraction of the polar herbicides into $KHCO_3$, further concentration of acids by methylene chloride extraction to a final volume of 1 ml, esterification with BCl_3/2-chloroethanol reagent, and EC-GC of the resultant 2-chloroethyl esters. A separate procedure for MCPA (4-chloro-2-methylphenoxyacetic acid) and MCPB [4-(4-chloro-2-methylphenoxy) butyric acid] in natural water at 0.1-0.2 µg/L levels is based on extraction from an acidified sample with methylene chloride, derivatization to pentafluorobenzyl esters, cleanup and fractionation on a silica gel column, and EC-GC determination. PCP is detected at 0.01 µg/L by benzene extraction from acidified water, partition into potassium carbonate solution, acetylation with acetic anhydride, partition into hexane, and EC-GC. Five N-methyl carbamates are determined at 0.10-1.0 µg/L levels by extraction from acidified water with methylene chloride, partition with base to remove phenols and acids present in the extract, hydrolysis with methanolic KOH to the respective phenols, extraction of the phenols with methylene chloride and derivatization with penafluorobenzyl bromide, cleanup and fractionation of the ether derivatives on a silica gel microcolumn, and EC-GC of the column eluates.

Cleanup is often not required for EC-GC analysis of surface water samples (163) and is usually not required for any type of water if a selective GC detector is employed. For example, the multiresidue analysis of 14 OP pesticides in natural waters has been carried out at ppb levels by extraction, concentration, and direct GC with a FPD detector in the P- and S-modes (119). Results of an interlaboratory study of the analysis of 15 water samples for 10 OCl pesticides without any column cleanup have been reported (164). Where needed, cleanup and separation of common chlorinated and OP insecticides extracted from water have been successfully carried out in silica gel microcolumns (165, 166) and columns of deactivated (5-20% H_2O) silica gel (above) and alumina (167).

Extracts of water, sediment, sludge, sewage, and soil often contain large amounts of elemental sulfur, which interfere in the GC analysis of early eluting pesticides with the EC or FPD detectors. Chemical desulfurization with Raney copper powder (168) or copper ribbon (169), precipitation with metallic mercury (170), reaction with CN^- (171), and treatment with tetrabutylammonium sulfite to produce an ion pair with sulfur as $S_2O_3^=$ (172) have been used to remove such interference. See also Subsection 9A,D).

Polar phosphorus, urea, and carbamate pesticides are extracted from water with more polar solvents such as chloroform or methylene chloride. Extraction of acidic or basic compounds is aided by adjusting the water sample to a controlled pH value. An XAD macroreticular resin can also be used for residue isolation and collection. Determination by GC is carried out using an appropriate selective detector after extract concentration and any required cleanup and/or derivatization steps. As an example, carbaryl and 1-naphthol have been determined in natural water at 2.5-10 ppb levels (82-102% recovery at 5 ppb) by EC-GC after methylene chloride extraction, cleanup on an XAD-8 column, and derivatization with heptafluorobutyric anhydride reagent (173). Sixteen organophosphorus pesticides were determined in drinking water at ng/liter levels by extraction with Amberlite XAD-2 resin, elution from the resin with hexane-acetone (85:15 v/v), and GC of the concentrated effluent using a nitrogen-phosphorus selective detector (174).

Chlorophenoxy herbicides and their esters have been determined by adjusting the water sample to pH 2, extracting with benzene or diethyl ether, methylating the acids with diazomethane or BF_3-methanol, followed by gas chromatography with an electron capture or microcoulometric detector (175) (see the footnote on page 6 concerning the hazards of diazomethane). PCP has been determined in marine biota and sea water by EC-GC of the amyl diazohydrocarbon derivative after Florisil cleanup (0.002 ppb) and by HPLC of the free phenol without cleanup (2 ppb) (176).

TLC determinations of carbamate, urea, triazine, and uracil herbicide residues in water have been reviewed (137, 177), as have the extraction, cleanup, GC determination, and confirmation of chlorinated insecticides in water and soils (178).

9A,D SOIL, HOUSE DUST, AND BOTTOM SEDIMENT

The analysis of soil and house dust for organochlorine pesticides is described in Section 11,A of the EPA PAM. Homogenized samples are Soxhlet-extracted with acetone-hexane, extract is concentrated in a K-D evaporator, and cleanup carried out on successive aluminum oxide and Florisil columns. Eluates are concentrated as required and determined by EC-GC. A similar AOAC method has been declared official final action for residues of aldrin, p,p'-DDE, p,p'-DDT, o,p'-DDT, p,p'-TDE, dieldrin, endrin, heptachlor, heptachlor epoxide, and lindane (179). Section 11,C of the EPA PAM references a procedure (180) for direct GC determination of carbamate pesticides in soils using Carbowax 20M-modified supports and the Hall electrolytic conductivity detector. This method is now being investigated by the EPA for possible future inclusion in the EPA PAM.

Sediment samples are partially air dried, mixed with sodium sulfate, and packed into a chromatographic column. The pesticides are extracted from the column by elution with hexane-acetone (1:1 v/v). The extract is washed with water to remove acetone, and the pesticides extracted from water with 15% methylene chloride in hexane. The extract is dried with sodium sulfate, concentrated to a suitable volume, and cleaned-up on a Florisil column. After desulfurization with copper, determination of organochlorine pesticides is by EC-GC. Details of the entire procedure are presented in Section 11,B of the EPA PAM. Air drying of the sample required 1-3 days, depending on the soil type. Such samples will contain at least 50% water. Pesticide concentrations are expressed on a "dry" basis, requiring determination of the dry weight of sediment by weighing a separate, air-dried sample before and after heating overnight at 100-110°C. Storage of soils in light can cause formation of artifacts of OCl pesticides (181). Moistening of dried soil with water (e.g., 80ml/300ml) may increase extraction of pesticides by solvents such as hexane-isopropanol (3:1 v/v) (182).

Sediment samples may contain elemental sulfur that will be recovered through the normal extraction and cleanup procedures for organochlorine and organophosphate pesticides and detected by the EC, FPD (P- or S-modes), and conductivity detectors. With the recommended GC columns and operating parameters, sulfur can completely mask the chromatogram from the solvent peak through the aldrin peak. The technique described in Section 11,B,VI of the EPA PAM for desulfurization employs vigorous agitation for one minute with bright metallic copper. Some pesticides may be degraded by this treatment (e.g., OPs, heptachlor), but these are not likely to be found in routine sediment samples because of breakdown in the aquatic environment. The procedure should be carried out if the presence of sulfur is indicated by an exploratory injection from the final extract concentrate or if sulfur crystallizes out when the 6 and 15% ethyl ether eluates from the Florisil column are concentrated. During determination of atrazine residues in soil containing high levels of ammonium nitrate fertilizers, the response produced by the N-thermionic detector was not constant for standards and samples due to the presence of the fertilizer in the sample extracts (183).

Part 5 of the 1979 Environment Canada Analytical Methods Manual, Inland Water Directorate, Ottawa, Canada, contains a method for organochlorine pesticides and PCBs in sediment and fish. Nineteen compounds are determined at 0.001-0.05 mg/kg levels by extraction of previously frozen samples with acetonitrile, partition with petroleum ether after appropriate dilution with water, and cleanup and separation into four fractions on a Florisil column. Each fraction is determined by EC-GC. Sulfur is removed by precipitation with copper powder or mercury.

Nine chlorinated insecticides were determined by a modified GC procedure (184) with recoveries of 75-99% from suspended sediment and bottom material. Extraction was with acetone and hexane added separately, coextractives (including PCBs) were isolated by alumina and silica gel column chromatography, and EC-GC was used to analyze the various column eluates. Some soil analyses have been carried out by EC-GC with no required column cleanup (185), but this is not common.

Shake (blending), Soxhlet, and column extraction methods were compared for efficiency in removing some twenty chlorinated insecticides from a sandy loam soil. There was no statistical difference among the three methods for the majority of pesticides, but shake extraction was significantly more efficient for BHC isomers (186). The shake extraction method with hexane-acetone after moistening the soil with 0.2 M NH_4Cl was studied collaboratively using standard AOAC analytical methods (Florisil cleanup and EC-GC) (187) and found to give excellent recoveries for six insecticides in three different soils (188).

Soil residues of chlorfenvinphos, chlormephos, disulfoton, phorate, and pirimiphos-ethyl were determined by GC with thermionic detection. Extracted compounds were cleaned-up on a carbon-cellulose column. Recoveries ranged from 95-101% (189). Another group of OP pesticides was determined in soil by GC with the thermionic detector, following extraction with acetone-hexane-benzene (1:1:1 v/v). Florisil was used to clean up and fractionate the residues. Dichlofenthion, chlorpyrifos, ethion, fonofos, and leptophos were eluted with benzene-hexane (9:1 v/v) and parathion, diazinon, chlorfenvinphos, malathion, phosmet, azinphos methyl, diazoson, and paraoxon with hexane-acetone (95:5 v/v) (190).

A multiresidue GC procedure for the herbicides dichlobenil, dinitramine, triallate, and trifluralin in soils was described by Smith (191). Extraction was carried out with acetonitrile-water (9:1 v/v) in a Sonic Dismembrator, herbicides were partitioned into hexane, and aliquots injected directly into an EC chromatograph. Recoveries were 92-107% from three soils at 0.05-0.5 ppm levels. Acetonitrile-water mixtures have proven to be especially efficient solvents for residues of herbicides of different chemical classes (192). Anilide herbicides were determined by GC after extraction from soil by blending with acetone (193). Urea and carbamate herbicides were recovered from soils by shaking with methanol (194) or acetone (195) and by alkaline hydrolysis and steam distillation (196). Iodinated (196) and 2,4-dinitrophenyl (195) derivatives were used for EC-GC determination of the herbicides. Triazines were extracted with diethyl ether from soil treated with ammonia (197) and uracils with 1.5 N NaOH (198). Nineteen acidic, neutral, and basic herbicides have been determined in soils by two dimensional TLC (199). Carbofuran residues in soil were determined at the 0.1 mg/kg level without cleanup by EC-GC after ammonium acetate extraction and formation of the dinitrophenyl-ether derivative (200). Uracils have been recovered by elution with water from a column prepared by mixing soil with Celite and $Ca(OH)_2$; the eluate was acidified and extracted with $CHCl_3$, and uracil determination was by RbCl thermionic-GC (201).

The electrolytic conductivity detector has been used to determine nitrogen-containing residues in crude soil extracts. A detector maintenance program for decontamination of the transfer lines and vent valve provided reliable operation with little "down time" even though lengthy extract cleanup was not carried out (202).

The drying and storage of soils can have an effect on residue analysis. For example, the extractable atrazine content of soil samples was reduced by drying at 45°C for 24 hours. Dried samples originally containing 1 ppm of atrazine showed no further significant loss when stored up to 180 days at room temperature, but there was significant loss between 180 and 360 days. Dried samples originally containing 10 ppm of atrazine showed significant loss after 90 days of storage (203).

The analysis of pesticides of many classes in soils and plants has been reviewed (204). Results of the U.S. EPA National Soils Monitoring program employing Florisil cleanup of extracts prior to EC- or FPD-GC for OCl and OP pesticides, and partition cleanup of extracts prior to GC determination of atrazine with an N-selective thermionic detector have been published (205).

POLYCHLORINATED BIPHENYLS (PCBs), OTHER COMPOUNDS

9A,E PESTICIDE-PCB MIXTURES

PCBs are among the most ubiquitous and persistent chlorinated pollutants found today in the environment. The residue analyst is concerned not only with the detection and quantitative estimation of PCBs but with their effect on the reliable determination of pesticide residues. PCB interference may occur with most common chlorinated pesticides in residue analysis, and the residue chemist must be aware of the nature of this interference with respect to the GC columns being used and their operating parameters. Interference in routine analysis is possible with p,p'-DDT, o,p'-DDT, p,p'-DDD, and p,p'-DDE, as well as with early eluting pesticides such as BHC isomers, aldrin, heptachlor, and heptachlor epoxide, since prominent PCB peaks have retention times similar to these pesticides on the recommended GC columns.

PCBs are frequently detected in human adipose tissues, often at concentrations similar to those of chlorinated pesticides, and interference with pesticide analysis can be significant, depending upon the columns and operating parameters used. These interferences demonstrate the nonspecificity of the electron capture GC detector and the need for careful confirmation by use of at least two GC columns, TLC, chemical reactions, etc. (Section 10).

9A,F APPEARANCE OF PCB CHROMATOGRAMS

Whenever an analyst observes a conglomerate of chromatographic peaks upon injection of a biological substrate into an EC detection system, the possibility of the presence of PCBs should be considered. For example, Figure 9-C shows a chromatogram resulting from the injection of 10 ng Aroclor 1254 on a 4% SE-30/6% QF-1 column operated at 200°C with a carrier flow of 70 ml/minute. The first isomer peak of consequence has an absolute retention of about 6 minutes and the final peak about 38 minutes. Figure 9-D represents the chromatogram of 6 ng Aroclor 1260 under the same conditions, and major peaks ranging from 8 minutes to nearly one hour are seen. Aroclors 1254 and 1260 have shown up most widely in a variety of environmental and tissue samples.

Figure 9-C. Aroclor 1254. Column 4% SE-30/6% QF-1, 200°C, carrier flow 70 ml/min.

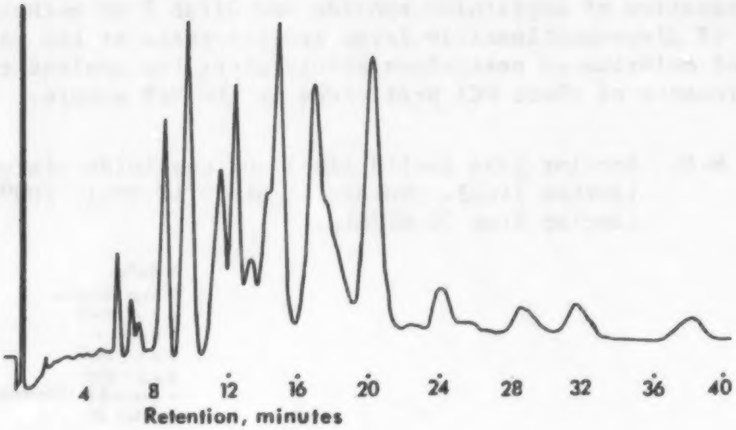
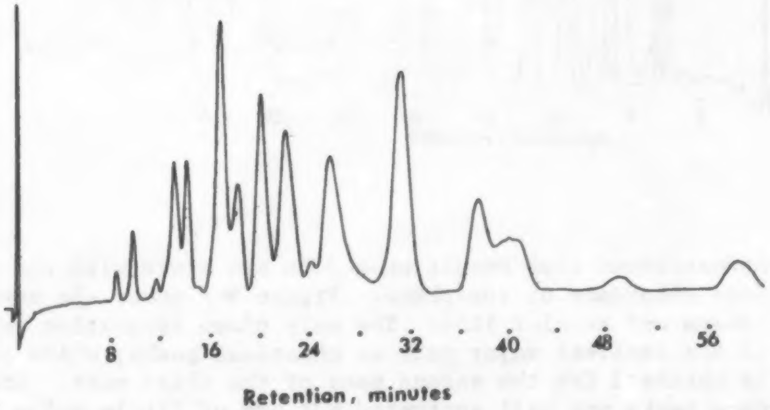
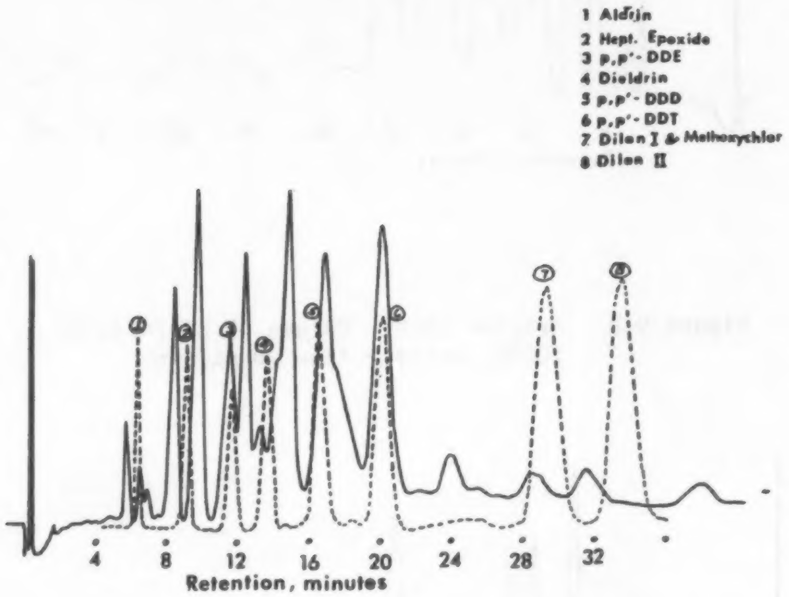


Figure 9-D. Aroclor 1260. Column 4% SE-20/6% QF-1, 200°C, carrier flow 70 ml/min.



The type of confusion evident when pesticides and PCBs are present in the same substrate is illustrated in Figure 9-E, showing Aroclor 1254 co-chromatographed with a mixture of eight chlorinated insecticides. Aldrin (peak 1), p,p'-DDE (3), p,p'-DDD (5), p,p'-DDT (6), Dilan I (7), and methoxychlor (7) are seen to overlap PCB peaks so closely that differentiation would be impossible. Heptachlor epoxide (2) and dieldrin (4) (in large quantities) are partially separated, while Dilan II is fairly well separated. A co-chromatogram of Aroclor 1260 with the same pesticide mixture would show good separation of aldrin and Dilan II, partial separation of heptachlor epoxide and Dilan I or methoxychlor, appearance of disproportionately large Aroclor peaks at the retention locations of chlorinated pesticides should alert the analyst to the possible presence of these OCl pesticides in the PCB sample.

Figure 9-E. Aroclor 1254 (solid line) and pesticide mixture (dotted line). Column 4% SE-30/6% QF-1, 200°C, carrier flow 70 ml/min.



Confusing chromatograms also result when PCBs are mixed with the multi-peak pesticides chlordane or toxaphene. Figure 9-F shows the co-chromatogram of chlordane and Aroclor 1254. The only clean separation is the first peak of the earliest major pair of chlordane peaks, while partial separation is obtained for the second peak of the third pair. The early minor chlordane peaks are well separated but are of little value for quantitation of chlordane. Aroclor 1260 does not interfere as seriously with chlordane under these same chromatographic parameters since the first PCB peak does not elute until after first two major chlordane peaks.

Figure 9-F. Aroclor 1254 (solid line) and chlordane (dotted Line) Column 4% SE-30/6% QF-1, 200°C, carrier flow 70 ml/min.

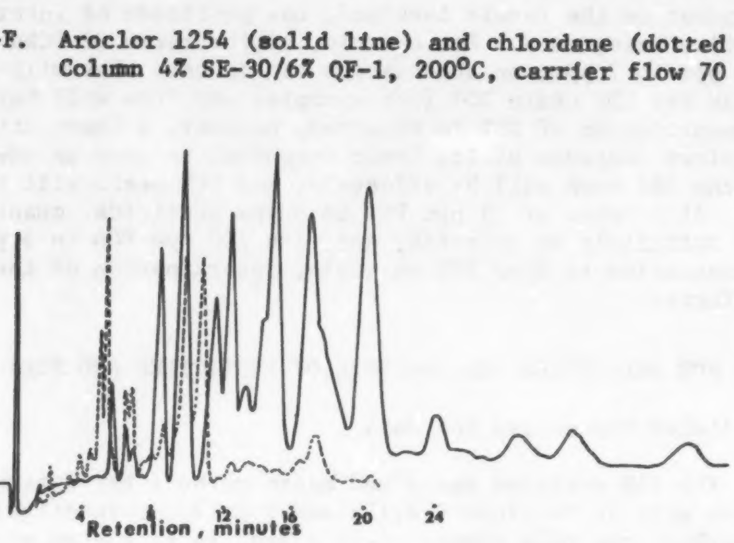
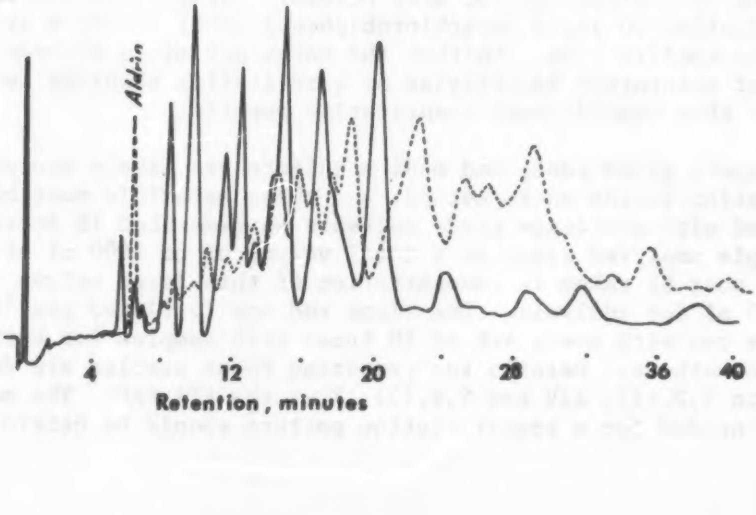


Figure 9-G shows a mixture of Aroclor 1254 with toxaphene. Analyses of toxaphene, chlordane, and PCBs are further confused because the chromatograms of environmental samples never exactly resemble those of standards. Chlordane is not very widespread in environmental samples, so its mutual analysis with PCBs is less likely to be a problem.

Figure 9-G. Aroclor 1254 (solid line) and toxaphene (dotted line) Column 4% SE-30/6% QF-1, 200°C, carrier flow 70 ml/min.



The actual effect of PCBs on quantitation of chlorinated pesticides is highly dependent on the levels involved, the pesticide of interest, and the attenuation being used. For example, if the ratio of PCBs to pesticides is 10 ppm to 3 ppm, an attenuation can be used that will give an adequate peak for DDE while DDT (for example) and PCBs will hardly be seen. If quantitation of DDT is required, however, a lower attenuation will be required (because of its lower response) to give an adequate peak size, the DDE peak will be off-scale, and PCB peaks will be more noticeable. At a ratio of 25 ppm PCB to 3 ppm pesticide, quantitation of DDT will definitely be affected, and with 100 ppm PCB to 3 ppm pesticide and attenuation to keep DDE on scale, determination of the latter would be affected.

9A,G METHODS FOR SEPARATION AND ANALYSIS OF PESTICIDES AND PCBs

a. Published Procedures and Data

The EPA PAM contains macro and micro methods for determining PCBs in human milk in Sections 9,B,(1) and 9,B,(2), respectively. In the macro method, the milk sample (4-24 grams) is extracted with acetone and hexane, PCBs are transferred to the hexane layer by adding sodium sulfate solution, and the hexane is dried by passage through a sodium sulfate column. Part of the sample is used for a lipid determination, and the rest is partitioned with acetonitrile and then fractionated on an activated Florisil column 10 cm in height. Identification and quantitation of PCBs is carried out by EC-GC and confirmation by use of different GC columns, and the electrolytic conductivity detector (Cl-mode), chemical derivatization by perchlorination, and GC-MS of pooled samples.

In the micro method, a 0.5 gram sample of milk is extracted with acetonitrile, residues are partitioned into hexane, the hexane is concentrated, and the PCBs are eluted through a 1 gram deactivated (3% water) Florisil column. The eluted PCBs are further separated from chlorinated pesticides on a micro silicic acid column. Chemical derivatization by perchlorination to yield decachlorobiphenyl (DCB) followed by EC-GC is used to confirm PCBs. Neither the macro nor micro methods are capable of accurately identifying or quantitating absolute levels of PCBs, but they provide semi-quantitative results.

Filter paper, glass wool, and sodium sulfate are likely sources of PCB contamination in the macro method, and these materials must be thoroughly precleaned with pesticide grade solvents as described in Section 3K. Each sample analyzed requires a total volume of ca 2000 ml of solvent, and care must be taken in concentration of this large volume to the final 1-5 ml for analysis. One blank and one fortified goat's milk sample should be run with every set of 10 human milk samples for both the macro and micro methods. Details for preparing these samples are described in Section 9,B,(1), XIV and 9,B,(2), X of the EPA PAM. The amount of Florisil needed for a proper elution pattern should be determined for each different lot by elution of analytical standards. Proper separation of PCBs and pesticides on the silicic acid column should be checked by chromatographing standard compounds and analyzing both eluate fractions. The Aroclor standard providing a chromatogram most closely resembling that of the sample should be used for quantitation of that sample.

Analysts inexperienced with the method should be guided through the procedures at least four times by a person experienced with the procedure, using duplicate samples already analyzed by the experienced worker. Then the analyst should be required to demonstrate proficiency on an additional set of four spiked samples without aid before handling actual samples.

The EPA Manual also describes the separation of PCBs from DDT and its analogs by the method of Armour and Burke (206) (Section 9,C), and a thin layer method for semiquantitative estimation of PCBs in adipose tissue (Section 9,D). Section 9,E illustrates chromatograms of different Aroclors on 4% SE-30/6% OV-210 or QF-1 and 1.5% OV-17/1.95% QF-1 GC columns, and Section 9,F tabulates relative retention values and response values of six Aroclors on OV-17/QF-1, SE-30/QF-1, and OV-210 columns. Retention indices have been calculated for all 210 possible individual PCBs on 13 GC phases, and recommendations were made for the best phase combinations for separations (OV-210, Apiezon L, and OV-225 were among the best single columns; OV-3 + CHDMS and OV-3 or OV-25 + poly MPE were the most discriminating pair) (207). HPLC and capillary column GC have also been used to separate PCB mixtures (208, 209).

Crist and Moseman (210) of the EPA reported a simplified micro perchlorination method for determination of PCBs in biological samples. A sample was cleaned-up by the modified MOG procedure (Section 7Aa), and the PCBs were perchlorinated with $SbCl_5$ to decachlorobiphenyl (DCB), which was cleaned-up by hexane partitioning and chromatography on a 1.6 gram column of activated Florisil. Details are in Section 9,B,(2),IX of the EPA PAM. The presence of impurities in $SbCl_5$ reagent that can cause erratic recoveries of PCBs was noted by Trotter and Young (211), and DCB impurity was detected in various brands of the reagents used in the Crist and Moseman procedure (210).

b. PCB Cleanup and Separation Systems

Depending upon the particular pesticides and PCBs present, the amounts of each, and the purpose of the analysis, it may or may not be necessary to separate PCBs and pesticides present in the same extract before the determinative step. Some combinations may permit quantitation of each without prior separation, others will require a separation before determination, and still others may require a separation procedure that destroys or converts some of the compounds to permit quantitation of those remaining unchanged.

PCBs are eluted with 6% ethyl ether-petroleum ether in the modified MOG procedure described in the EPA PAM, Section 5,A,(1),(a) and in the FDA

PAM multiresidue procedures, Sections 211 and 212. They elute with eluant 1 of the alternative methylene chloride elution system (Section 9A,B of this Manual and Section 252 of the FDA PAM). A study by Lieb and Bills (212) found that the storage temperature of Florisil after initial activation (overnight, 130°C) influenced the GC pattern obtained for Aroclor 1254 separated from lipids on a column of the Florisil. To avoid selective adsorption of some PCB components and erroneous PCB analyses, storage of activated Florisil at room temperature was recommended. This, however, is in opposition to the procedure recommended for routine pesticide work (continuous storage at 130°C until use) and should be studied further. Hydroxy PCB metabolites extracted from cow's milk were cleaned up by extraction with aqueous alkali and re-extraction of the acidified aqueous solution with organic solvent prior to further TLC cleanup and GC-MS determination (213).

The method of Armour and Burke (206) has been most used for pesticide-PCB separation. The 6% ethyl ether-petroleum ether Florisil column eluate or eluate 1 of the alternative procedure (Section 9A,b of this Manual) is concentrated to an appropriate volume and a 5 ml or smaller aliquot applied to a column of partially deactivated silicic acid and Celite, standardized before use to effect the best possible separation between p,p'-DDE and Aroclor 1254. Petroleum ether followed by acetonitrile-hexane-methylene chloride (1:19:80 v/v) are used to elute the column, both fractions being collected in a K-D evaporation flask. The eluates are concentrated and subjected to EC-GC. Mixed results have been reported with this silicic acid separation system. PCBs and polychlorinated terphenyls split between the two fractions (EPA PAM, Section 9,C, Table 1) as do the pesticides aldrin and p,p'-DDE (Canadian PAM, Section 7.5). Polychlorinated naphthalenes (214) and dioxins elute in the first fraction and most other chlorinated pesticides (e.g., chlordane, toxaphene, DDT, heptachlor, lindane) in the second. Because of the division of some compounds between the two eluates, GC columns must be carefully chosen to separate the components present in each fraction. The tables of relative peak heights and peak retentions in the EPA PAM can help in this selection. The chemist running this procedure for the first time should perform a sufficient number of recovery trials with spiked samples to gain confidence in its reliability. Impurities present in silicic acid adsorbent batches, their effect on separations, and means for their removal have been described (215). Pesticide-PCB separations were found reproducible only for individual batches of adsorbent. Porter and Burke have reported the separations of TCDD from PCBs on acidic, basic, or neutral aluminum oxide; PCBs were eluted with hexane-methylene chloride (99:1 v/v) and di, tri-, and tetrachlorodibenzo-p-dioxins with hexane-methylene chloride (80:20 v/v) (216).

A slightly modified version of the Armour-Burke method is detailed in the Canadian PAM Section 7.5, and the method has been miniaturized for determining chlorinated pesticide and PCB residues in fish. In the latter method, the sample is dried with Na_2SO_4 and packed into a column, which is eluted with petroleum ether. Cleanup and separation of the extract is on 1 cm (id) Florisil and silica gel columns (217).

Other columns used in various multiresidue cleanup procedures provide at least partial separations of organochlorine pesticides from PCBs. These include columns of activated alumina (67; Section 90); silica plus alumina (64; Section 90); 60 A° silica gel eluted with pentane (elutes PCBs and mirex) and benzene (recovers DDE, DDT, TDE, BHC, dieldrin) (218); and charcoal (219, 220). Elution of a charcoal column with diethyl ether-acetone (3:1 v/v) removes OCl pesticides while PCBs are then eluted with benzene (59, 221). A Norit C-170 charcoal-polyurethane foam (40:60 w/w) mixture is especially useful for separation of mirex and photomirex from PCBs (222).

Section 251.2 of the FDA PAM describes derivatization and micro-column chromatography procedures for removal of DDT and related compounds from extracts containing PCBs. Cleaned-up extracts are treated with alkali to convert DDT to DDE and TDE to its olefin; PCBs are unchanged. Subsequent oxidation of the solution with chromium trioxide in acetic acid converts DDE and TDE olefin to dichlorobenzophenone, but again PCBs remain intact. PCBs are then separated from polar dichlorobenzophenone by elution with petroleum ether from a micro activated Florisil column. Dichlorobenzophenone is eluted, if required, with ethyl ether-petroleum ether (1:1 v/v). Recoveries of Aroclors 1242, 1254, and 1260 ranged from 77-100% (0.4-56 μg amoumts), while DDT, DDE, and TDE were recovered (as dichlorobenzophenone) between 5-86% (2-33 μg). The same reactions used in this GC determinative procedure have been applied to TLC estimation (Subsection d.) and confirmation (Subsection e.) of PCBs.

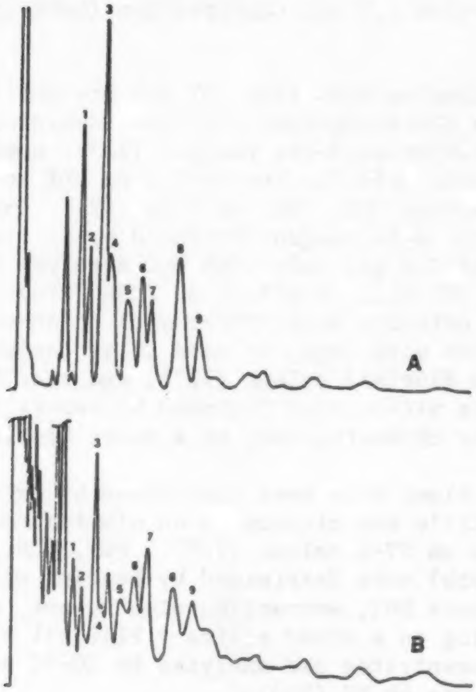
Other techniques for separating PCBs from DDT and its analogs by chemical derivatization and column chromatography include: dehydrochlorination with 1,5-diazobicyclo(5.4.0)undec-5-ene reagent (223); sodium dichromate in acetic acid plus sulfuric acid for conversion of DDE to dichlorobenzophenone without affecting DDT, TDE, or PCBs (224); oxidation by chromic acid glacial acetic acid reagent followed by silica gel column chromatography (225); a silica gel tube with MgO catalyst for conversion of DDT to DDE and TDE to DMU without effect on PCBs (226); heating cleaned-up fish or serum extracts with KOH/ethanol to convert OCl pesticides to alkenes, oxidation with Cr_2O_3 to more polar compounds, and separation from PCBs on a Florisil column (227); and reaction of fish tissue extract with fuming nitric acid followed by separation of nitrated PCBs from mirex analogs by chromatography on a micro Florisil column (228).

Aroclor 1254 residues in blood have been determined by extraction with hexane-saturated acetonitrile and cleanup on an alumina column. Eluates were analyzed by EC-GC on an OV-1 column (229). PCT, PCB, and DDT residues in blood (5-11 ppb) were determined by heating with ethanol and KOH to dehydrochlorinate DDT, extracting with hexane, washing with H_2SO_4, and chromatographing on a mixed silica + Florisil + Na_2SO_4 column. The hexane eluate was concentrated and analyzed by EC-GC on a 2% OV-1 column, and confirmation was by MS (230).

c. GC Quantitation of PCBs

One of the most difficult aspects of PCB quantitation is to obtain a match between the sample and a standard. Because individual congeners in the original source are likely to be distributed differently due to varying volatilities, solubilities, and reactivities, biological and environmental samples seldom have a GC peak pattern that will exactly match any Aroclor standard, and even commercial preparations of PCBs vary in abundance of minor components from batch to batch. In addition, detector response of different PCB isomers can vary by as much as 10,000 fold, so that any similarity between samples and a known commercial PCB mixture is likely to be purely fortuitous. For example, the upper chromatogram in Figure 9-H is that of a standard PCB mixture, Aroclor 1242. Below it is the same mixture added to an ambient air sample at a level equivalent to 100 ng/m^3. The PCB mixture

Figure 9-H. Gas chromatograms of Aroclor 1242. (A) standard fortification solution diluted 10 times to 200 pg/µl; (B) residue in upper foam trap after 24 hours at 225 L/minute. Numbers indicate peaks used for quantitation.

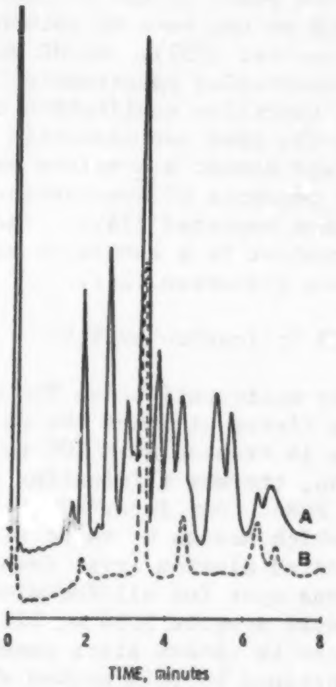


was added to the air stream as vapor while the sample was being collected, resulting in alteration of the relative peak ratios because the various components were trapped with efficiencies ranging from 40-95% and also because of contributions from background materials. It would be difficult to identify the added PCBs in this sample from GC data alone. Figure 9-I shows an even more difficult, but no less typical, case. Chromatogram A is Aroclor 1016, while B is what was isolated from the brain of a rat that had been fed this PCB mixture for one year (231). The problem of accuracy in PCB analysis is not easily solvable. Because of the complexity of commercial mixtures, identification of individual components is not practical. Complete separation by GC is impossible, even with capillary columns. The mass spectrometer cannot usually distinguish between all PCB isomers.

The most widely used practical approach for PCB quantitation is to compare the total area or height of detector response for the residue peaks to the total area or height of response obtained under the same conditions for a known weight of the commercial Aroclor standard with the most similar pattern. Only those peaks from the sample that can be attributed to chlorobiphenyls are used, and these peaks must also be present in the chromatogram of reference materials. If the presence of more than one Aroclor is clearly indicated, the residue may be quantitated using mixtures of Aroclor standards judged appropriate for different portions of the sample chromatogram. In one interlaboratory study of PCB analysis using Aroclor 1254 as reference standard, quantitation via the three specific peaks with retention times, relative to DDE, of 127, 147, and 177 produced the best interlaboratory agreement (232).

Figure 9-I.

Electron capture gas chromatograms of (a) Aroclor 1016 standard and (B) PCB residue extracted from brain of rat fed on diet containing Aroclor 1016 for one year.



The procedure of Webb and McCall (233) has an advantage in that residues can be quantitatively measured on a GC peak-to-peak basis against a series of reference Aroclors with known weight percentage compositions for individual peaks. Reference Aroclors 1016, 1242, 1248, 1254, and 1260 have been characterized using a Hall electrolytic conductivity detector for Cl measurement and chemical ionization MS with single ion monitoring for molecular weights (234). A ten laboratory collaborative study of PCB quantitation in synthetic standard mixtures, milk, and chicken fat samples indicated that greater overall precision was possible using the individual peak method compared to total area or height (235). The individual peak method has been made official first action by AOAC as an alternative to the total area quantitation procedure (56).

All PCB components may be converted by perchlorination with $SbCl_5$ to a single derivative (decachlorobiphenyl), and the total PCB content may then be measured as this compound (210, 236; Subsection 9A,Ea). Quantitative data are not truly valid with this approach unless the average chlorine content of the original PCBs in the sample before chlorination is known. A related approach to quantitation is dehydrodechlorination of PCBs to biphenyl by lithium aluminum hydride in dodecane, followed by HPLC with a UV detector at 248 nm. The absolute detection limit was 100 ng, and DDT isomers, chloronaphthalenes, and PCTs were determined simultaneously (237).

Other quantitation approaches that have been attempted include estimation of the weight of PCB injected by dividing the retention time x peak height for all PCB peaks by the product of peak height and retention time for 1 ng p,p'-DDE on the same GC column (238), and peak resolution and matching by a computer (239). GC-MS with individual mass monitoring using a minicomputer-controlled spectrometer has been reported (240). This method provided sensitive qualitative and quantitative analysis of sediment extract without the need for elaborate column adsorption separations prior to GC. Beroza and Bowman's p-values have been applied to quantitation of p,p'-DDT in the presence of non-resolved PCB peaks, and results within 11% of actual were reported (241). The USFDA approach to chemical profiling of PCB content in a sample to select the most suitable quantitation standard has been discussed (242).

d. PCB Estimation by TLC

The semiquantitative TLC procedure (243) for determination of PCBs in adipose tissue utilizes the 6% eluate of the Florisil cleanup. The concentrate is treated with KOH to dehydrochlorinate DDT and DDD to their olefins, thereby eliminating the problem of separating the pesticides from the PCBs. Any interfering DDE is then oxidized to p,p'-dichlorobenzophenone, which has an R_F value different from the PCBs on a silver nitrate-impregnated alumina layer developed with 5% benzene in hexane. The PCBs give one spot for all formulations, and this is quantitated against a standard Aroclor 1254 or 1260 spot run on the same plate. The best standard can be chosen after examining a preliminary GC trace. The final values obtained by this method should be considered as approximations, with a precision of roughly ±50% indicated by recovery studies. The minimum level of detection is ca 1 ppm by exposure of the layer to UV light. An EPA human monitoring program for PCB levels in adipose tissue has utilized this TLC procedure with confirmation by combined GC-MS (244).

e. Confirmation of PCBs

Confirmation of PCBs has been obtained by perchlorination (210, 236), alkali treatment (245), and reaction with chromic acid (chromium trioxide). The stability of PCBs to alkali is useful for confirming the identity of PCB residues, and at the same time, conversion of DDT to DDE by the alkali removes some interference to quantitation of PCBs. Treatment with alkali also provides additional cleanup for many sample types. Resistance to oxidation with chromic acid-acetic acid reagent is also useful evidence for identifying PCBs in the presence of reactive pesticides such as DDE and DDT (243) and chlorinated naphthalenes (246). However, it has been reported (247) that alteration of PCB chromatographic patterns can occur upon chromium trioxide acid digestion of animal tissue extracts, including changes in peak areas and disappearance of several PCB homologs.

Two-dimensional (248) or multi-development reversed phase (249) TLC systems, which separate PCBs from DDE, DDT, and other pesticides, can aid identification. PCBs are destroyed by UV irradiation, but many pesticides may be altered as well (250). Toxaphene survives UV treatment that destroys PCBs and can be confirmed in mixtures in this way. Mirex, a late eluting pesticide that usually is not interfered with by PCBs, also withstands UV irradiation and can thus be confirmed. Irradiation with controlled UV wavelengths has provided identification and determination of aldrin, dieldrin, heptachlor, and heptachlor epoxide in mixture with PCBs. Photoisomerization reactions of the pesticides, producing products with longer retention times, were induced with wavelengths >290 nm; subsequent irradiation with wavelengths >230 nm yielded photodechlorinated products of PCBs with shorter retention times (251). Most organochlorine pesticides are destroyed by reaction with HNO_3-H_2SO_4 whereas PCBs and toxaphene are unaffected. Chlorinated pesticides were selectively detected in the presence of PCBs by use of a modified Coulson conductivity detector at 600°C with a hydrogen flow of 1-2 ml/min. (252). Mirex and PCBs have been differentiated based on the low sensitivity of the Hall detector for the latter (253). A collection of spectra helpful in confirming isolated residues of PCBs has been published (254).

9A,H DETERMINATION OF POLYBROMINATED BIPHENYLS

Polybrominated biphenyls (PBBs) were manufactured for use as a fire retardant from 1970 to 1973. Since the summer of 1973, when PBBs were accidentally mixed with dairy feed resulting in the contamination of livestock and food products, the sensitive determination of low levels of PBB residues has been of interest to the FDA and the EPA. One commercial PBB fire retardant (Firemaster BP-6) has been chemically and toxicologically evaluated; 13 different polybromobiphenyls were found plus a brominated naphthalene contaminant, and biological effects were ascribed to PBBs (255).

A rapid method has been developed for analysis of plasma, feces, milk, and bile using all disposable glassware to reduce the amount of laboratory background and cross-contamination of samples (256). The authors found that this type of equipment was necessary because methods that had proven to be effective for decontamination of PCBs were not effective for PBBs. The methodology involved multiple extraction of ethanol-denatured sample (except for feces) with petroleum ether-diethyl ether (1:1 v/v) in a disposable test tube, followed by cleanup on a miniature adsorption column packed in a 23 cm disposable Pasteur pipet. The column contained Florisil, silica gel, and sodium sulfate in different proportions, depending on the sample. The column was eluted with 5 or 10 ml of petroleum ether-benzene (98:2 v/v) into a disposable screw top vial. Determination of PBBs in the concentrated eluate was made by EC-GC on a silanized 5% OV-17 column. Mean recoveries for the six major components of a commercial PBB mixture were approximately 96% for plasma, 59% for feces, 98% for milk, and 89% for bile at 0.05-50.0 ppm levels. The maximum background level was 0.0007 ppm for the major hexabromobiphenyl peak, corresponding to a minimum detectable limit of ca 0.001 ppm.

The separation and characterization of PBBs by chromatography and spectroscopy have been studied (257). Columns containing 1% SE-30 or 2% OV-17 were used for GC-FID-MS, 5 μm silica gel 60 (Merck) columns for HPLC (UV detection), and paraffin-coated kieselguhr for reversed phase TLC. In addition to quadrupole MS, NMR and UV spectroscopy were evaluated. Capillary GC has been used to separate PBBs, as well as chlorinated dibenzofurans and anisoles (258).

PBB residues in dairy products were determined at 7 ppb levels by co-extraction from the sample along with fat, separation by GPC, EC-GC on an OV-101 column, and confirmation by TLC (259).

9A,I SEPARATION AND DETERMINATION OF POLYCHLORINATED TERPHENYLS

Polychlorinated terphenyls (PCTs) are also recovered by the multiresidue procedure described in Section 5,A,(1),(a) of the EPA PAM, but these compounds elute from the GC column much later than OCl pesticides and PCBs and, therefore, do not interfere. To determine PCTs, GC parameters must be altered to provide more rapid elution and greater sensitivity. The spectrometric and GC properties of 22 PCTs have been reported (260).

PCTs were identified at trace levels in pooled samples of human adipose tissues by gas chromatography-mass spectrometry. The extracts were cleaned-up by the modified MOG procedure (Section 9Aa) and then gel permeation chromatography on BioBeads SX-3 eluted with ethyl acetate-toluene (3:1 v/v). GC-MS analyses to confirm the polychlorinated terphenyl residues were made using a 152 cm x 2 mm id glass column packed with 3% OV-1 on Gas Chrom Q. The column oven was programmed from 150°C (1 minute) to 300°C at 4°C/minute. Mass spectra were acquired over the range 420 to 720 amu at 6 seconds/scan for PCT confirmation (261).

9A,J SEPARATION AND DETERMINATION OF DIOXINS

Section 9,G of the EPA PAM contains sample preparation and capillary column GC-MS techniques developed and currently applied by EPA laboratories for isolation, detection, quantitation, and confirmation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) residues (262). Tissues, milk, water, soil, and sediment samples are subjected to an "acid-base" sample preparation involving saponification with hot caustic solution, followed by extraction with hexane, washing with concentrated sulfuric acid, cleanup by alumina column chromatography, and capillary column GC-high resolution mass spectrometric multiple ion selection analysis for TCDD residues. Fish tissue is subjected to a "neutral" cleanup procedure in which extraction is carried out with acetonitrile, and cleanup by solvent partitioning and Florisil column chromatography precedes alumina column chromatography. ^{37}Cl-TCDD is added to all samples as an internal standard or marker to monitor and determine the cleanup efficiency. Sensitivity of the procedure is in the 0.02-100 ppt concentration range. Extreme care and very clean laboratory practices are mandatory for low ppt analyses.

The efficiency, accuracy, precision, and validity of ppt TCDD analyses depend on an incorporated quality assurance program that is described as part of the procedure in Section 9,G of the EPA PAM. It is important that TCDD analyses be conducted only by trained personnel with strict safety procedures in effect. The hazards and analysis of TCDD have been reviewed (263).

Reports from laboratories that have conducted environmental monitoring projects for TCDD and have developed and applied analytical cleanup systems and mass spectrometric methods of analysis for ppt levels of TCDD residues in environmental, biological, human, and agricultural samples and chemical formulations are contained in references (264-275). It has been shown (276) that analysis of environmental samples by low resolution GC-low resolution MS alone is acceptable if suitable control samples are available to show the absence of interferences. When suitable controls are not available and when cleanup is nonspecific, positive results for TCDD must be confirmed by high resolution MS, preferably using mass fragmentography with single or multiple ion detection and/or chemical ionization (277). A recent HPLC method (278) shows promise of being specific for TCDD and eliminating the need for high resolutions MS confirmation.

Chlorinated benzyl phenyl ethers have been identified as a possible serious interference in the GC-MS determination of chlorinated dibenzo-p-dioxins. These compounds, which have been extracted from 2,4,5-trichlophenol, have retention times and MS responses similar to TCDDs (279).

9A,K DETERMINATION OF ETHYLENETHIOUREA (ETU)

Because of its toxicological significance, constant occurrence as a terminal residue following crop treatments with ethylenebisdithiocarbamate fungicides, and its actual presence in technical ethylenebisdithiocarbamates, analytical methods for determination of ETU have become extremely important. A method for ETU in apples (280) was based on reaction with benzyl chloride to give 2-benzylmercaptoimidazoline, which is subsequently treated with trifluoroacetic anhydride to yield 2-benzylmercapto-N-trifluoroacetylimidazoline. This derivative is measured by EC-GC.

ETU residues were measured in various crops by methanol extraction, alumina column cleanup, and derivatization with 1-bromobutane in the presence of DMF and sodium borohydride. The resulting 2-butylmercaptoimidazoline was measured down to 0.01 mg/kg with an FPD detector (281). A similar method that determines ETU in milk, fruits, and vegetables as the same derivative has been collaboratively studied (282) and recommended as an AOAC official first action method (283).

EC-GC as well as S-mode FPD-GC have been used to determine ETU residues from crops after derivatization with m-trifluoromethylbenzyl chloride (284). The trifluoroacetylated S-benzyl derivative has also been used to determine ETU residues on tomatoes (285). ETU residues on fruit and vegetable crops were determined at 0.01-0.1 ppm levels without derivatization (286). After methanol extraction and cleanup by hexane/aqueous NH_4Cl partition and alumina column chromatography, GC was performed on a 3% Versamid 900 column with S-mode FPD detection. Recoveries ranged from 62-95%.

The occurrence, chemistry, and metabolism of ETU and analytical methods for its determination have been reviewed (287).

9A,L DETERMINATION OF CONJUGATED PESTICIDE RESIDUES

Pesticides and pesticide metabolites are known to form carbohydrate (glycoside, glucuronide), amino acid, sulfate, alkyl, and acyl conjugates in various plant, animal, and soil systems. Because of the potential biological activity of many of these conjugates, their identification and determination has become an important task for the pesticide analyst.

Because conjugates are, in general, more polar and less lipophilic than the parent pesticides, analytical methods are designed to take into account these differences. In addition, the lability of certain conjugates may dictate the analytical approach taken when isolating and identifying the intact compound or a derivative, e.g., the need for protection of labile moieties from hydrolysis during extraction or the choice of column LC rather than GC for separation of thermally unstable or nonvolatile conjugates. Analysis of enzymatic or chemical hydrolysis products is useful confirmatory information for conjugates that have been identified intact or may serve for the quantitation of a conjugate residue.

Different types of mass spectrometry (Section 10), including electron impact, chemical ionization, field desorption, and laser ionization, are probably the most powerful and widely used tools for structural analysis of conjugates. The field desorption method is especially useful due to its applicability to polar materials. NMR, particularly using proton nuclei with the sensitive Fourier transform technique, is another important aid for structure elucidation (Section 10K), as are traditional IR and UV absorption spectrometry and micro-IR (Section 10J).

Specific isolation methods depend on the exact nature of the conjugate of interest and the sample matrix. Most conjugates are extractable with water, alcohol, and water-alcohol mixtures from insects, plants, or tissues. Samples may be freeze-dried and pre-extracted with an organic solvent to remove lipophilic materials. Purification, separation, and concentration of conjugates have been carried out using simple solvent partitioning, counter-current liquid-liquid distribution, extraction with liquid anion-exchangers, Amberlite XAD-2 polymer columns, silicic acid columns, Porapak Q resin columns, Sephadex LH-20 gel columns, DEAE-cellulose and DEAE-Sephadex anion-exchange columns, Sephadex G gel columns, Biogel P columns, cation-exchange resin amino-acid analyzer columns, liquid anion-exchange paper chromatography, TLC, and GC of conjugates either directly or after forming a volatile derivative.

Most analytical work on pesticide conjugates to date has been conducted for structural identifications or metabolism studies. The usual radiotracer detection techniques are widely used in metabolism research. A review of analytical methods for different conjugate types, including many literature references, and examples of applications to different research problems will be found in reference (288). This volume also contains information on the nature and analysis of "bound" or unextractable pesticide residues. One approach to the analysis of bound residues was reported for chloroaniline bound to lignin fractions of plants based on release by pyrolysis (289); pyrolysates containing intact chloroanilines were collected and derivatized as trifluoroacetanilides, which were purified and determined by EC-GC.

Relatively little attention has been given to the recovery of pesticide conjugates by analytical procedures designed to determine the parent residues. When the problem is addressed, the usual approach is that which was taken to determine PCP residues in urine (24). The analytical procedure for intact PCP residues was modified to include an acid hydrolysis, the purpose of which was to free the conjugated forms of the pesticide and allow its derivatization along with the unchanged parent compound (see Section 9E).

A few similar procedures for other pesticides have been published, the hydrolysis step in some cases serving both to break the conjugate and to hydrolyze the parent pesticide to a new form prior to determination (e.g., hydrolysis of carbamate insecticides to the corresponding phenol, which is derivatized, cleaned-up, and determined by GC). 3-Hydroxy-carbofuran, the major carbofuran metabolite produced in animals, is present as the water soluble glucuronide conjugate. A mild acid hydrolysis was used to free the conjugated form of the metabolite and allow its extraction with organic solvent along with the parent compound (290).

Conjugates of 2,4,5-T in biological samples have been broken and the free acids released by a basic hydrolysis step (291). Residues of conjugated iodofenphos phenol metabolites were recovered from liver and kidney tissue by extraction with ethanol-water-1N sodium hydroxide (90:10:1 v/v), hydrolysis with 1N sulfuric acid, and hexane + ethyl ether extraction (292).

9A,M REVIEWS OF ANALYTICAL METHODS FOR PESTICIDES, PCBs, AND OTHER NON-PESTICIDE POLLUTANTS

See Subsection 1G in Chapter 1 for a general bibliography of important books and reviews on the analysis of pesticides and related pollutants.

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Section 10

CONFIRMATORY AND OTHER DETERMINATIVE PROCEDURES

10A REQUIREMENTS FOR POSITIVE CONFIRMATION OF PESTICIDE IDENTITY

Obtaining convincing identification of a trace residue is a major task of the pesticide analyst. The identity of pesticide residues should always be confirmed by a method different from that used in the initial determination since interpretation of results (e.g., decisions of a legal or health nature) as well as reliable quantitation (selection of standards) depend on correct identification. Multiresidue GC analytical methods do not provide irrefutable identification since interfering materials and artifacts are often observed, and metabolic and decomposition products may be encountered.

A specific example of a serious identification problem is the determination of the PCBs, which are easily mistaken for pesticide residues such as p,p'-DDE and p,p'-DDT. Another important example concerns overlapping peaks when foods are screened for tolerance levels: a 4% SE-30/6% QF-1 column may give peaks at essentially identical retention times for endrin and o,p'-DDT, for Endosulfan I and p,p'-DDE, and for β-BHC and lindane. Both DDT and DDE are very common pesticides with rather high tolerance levels. Thus, if the analyst is unaware that endrin and endosulfan may produce corresponding GC responses, he may conclude that observed peaks indicate only insignificant quantities of DDT and DDE relative to tolerance levels and that no further work is necessary. Unfortunately, what appears to be insignificant response for DDT and DDE is very substantial response for endrin and Endosulfan I because of lower GC sensitivity to these compounds and lower tolerance levels; therefore, confirmation of identity is mandatory (1).

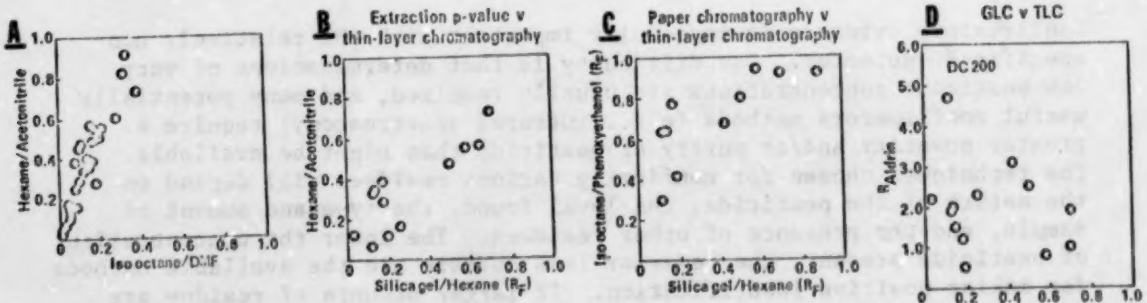
Confirmatory evidence is especially important with the relatively nonspecific EC detector. One difficulty is that determinations of very low pesticide concentrations are usually required, and many potentially useful confirmatory methods (e.g., infrared spectroscopy) require a greater quantity and/or purity of pesticide than might be available. The techniques chosen for confirming various residues will depend on the nature of the pesticide, the level found, the type and amount of sample, and the presence of other residues. The lower the concentration of pesticide present, the fewer or less certain are the available methods for making positive identification. If larger amounts of residue are found and can be isolated in a reasonably pure state, infrared (IR) spectroscopy and mass spectrometry can provide unequivocal identification.

Considerations of set theory (2) indicate that three independent "equivocal" results are required in order to be confident of the identity of a pesticide residue. These might be elution in a certain fraction from a liquid chromatography cleanup column, a GC retention time, and a positive response of a selective GC detector. Another possible combination that would be a basis for confidence is the GC retention times from a polar column and a nonpolar column plus an R_F value from paper chromatography (PC) or thin layer chromatography (TLC) or an extraction p-value. Still another would be a GC retention time, a PC or TLC R_F value, and the GC retention time of a derivative formed by a chemical or photochemical reaction.

The dependence or independence of measured values was studied by Elgar (3) who reported that many widely used confirmatory methods may not give truly independent evidence of identity since they are measuring the same chemical or physical properties. Thus, care must be exercised when deciding which methods to use in combination to avoid doing a great deal of work without gaining additional useful information. Examples of highly correlated (not independent) values include GC retention times on certain stationary phases (Figures 5-A,A in Section 5); PC or TLC R_F values from certain adsorbent/solvent systems; p-values in different solvent pairs; and PC, TLC, and p-values. These combinations will not provide independent information for confirming residue identity.

In Figure 10-A, the correspondence between extraction p-values in hexane/acetonitrile and isooctane/DMF solvent pairs (A), and p-values in hexane/acetonitrile and TLC R_F values with the system silica gel/hexane (B) is shown by the generally straight line along which the plotted data points lie. The independence of TLC and PC data [Figure 10-A, (C)] and GC and TLC data (D) is illustrated by the scatter of the points. Clearly, many combinations of alternative columns, selective detectors, p-values or PC or TLC, and chemical derivatization can be applied for purposes of confirmation.

Figure 10-A. Degree of correspondence between different types of data for residue confirmation. A = extraction p-values, B = TLC vs p-values, C = PC vs TLC, D = TLC vs GC



When the analyst is making pesticide identifications, common sense is necessary. An example of misapplied common sense would be reporting methyl or ethyl parathion in human fat; metabolically it is virtually impossible for parathion to persist per se and to appear in a tissue or body fluid (except gastrointestinal). The persistence of heptachlor would also be very unlikely because body metabolism normally converts it to heptachlor epoxide. Chromatography with EC detection of human adipose tissue from the general population often produces peaks with retention characteristics very close or identical to the RRT_A values for α-BHC and/or o,p'-DDE. However, the presence of these compounds has rarely, if ever, been confirmed. In these instances, the peaks in question represent artifacts that happen to have the same retention times as these pesticides, and careful confirmation by ancillary techniques would provide the proper identification.

In summary, since all methods and tests regularly used in residue analysis are presumptive in nature (the behavior of an unknown is compared to that of a known, standard material), it is most desirable to use a number of tests that measure different chemical or physical properties. The initial GC method should have been proved to recover and detect the pesticide residues of interest, and it is desirable that data are available on the behavior of many pesticides and their metabolites and degradation products in the various operations that comprise the method. The analyst should be familiar with and capable of fully using and interpreting these data and all other available information, including pesticide usage, the chemistry and metabolism of residues, common artifacts from sample substrates and reagents, and the possibility of interfering residues, such as PCBs and phthalate esters. Analytical conclusions must be reached with an open mind, common sense, and reasonable judgment. The extent of confirmatory effort and the exact procedure chosen will depend on factors such as the history and significance of the sample; nature and level of the residues; sample type; purpose of the analysis; and practical considerations such as time, cost, number of samples, and available instrumentation. Alternatives to confirmation of residues in all samples are discussed in Section 1E. "Unusual" residues should be verified in all analyses, even at low levels, to support a decision to devote further effort to tracing their origins. Confirmatory methods should yield identical results with both the suspected sample residue and standard reference material subjected concurrently to the same tests. Similar concentrations of the sample and standard should be used in the comparative testing to demonstrate quantitative as well as qualitative confirmatory evidence (FDA PAM, Section 601). The following subsections discuss the more widely used confirmatory procedures, some of which are also useful for residue quantitation.

10B GC RELATIVE RETENTION TIMES

In most laboratories, the initial, tentative identification of a pesticide residue results from a multiresidue procedure involving extraction, cleanup, and gas chromatography. Tables of GC retention times for particular column-detector combinations are normally used for the tentative identification. The recovery of a residue through the preliminary cleanup steps should not be overlooked as valuable, supplemental confirmatory evidence. This is particularly true when such characteristic properties as the ability to withstand acid or alkali treatment or elution in a particular fraction from an adsorbent column is involved.

The following guidelines are useful for the proper utilization of retention times in making compound identifications.

a. The use of relative retention or Kovats' retention indicies (4) rather than absolute retention is more reliable (Subsection 5N in Section 5).

b. Be highly suspicious of any peak with a calculated relative retention value (RRT) that does not precisely match that of the standard or that of the tables [EPA PAM, Section 4,A, (6)]. A simple aid is to co-chromatograph some pure standard of the suspect compound along with the sample extract and observe the peak configuration compared to that of the sample alone. If some distortion is evident in the configuration of a given suspect peak, the identification can be safely negated.

c. If cleanup is used on the sample, always run the elution fractions separately. Do not pool the elution cuts. Selective adsorption combined with GC retention characteristics provides a valuable identification tool for pesticide analysis.

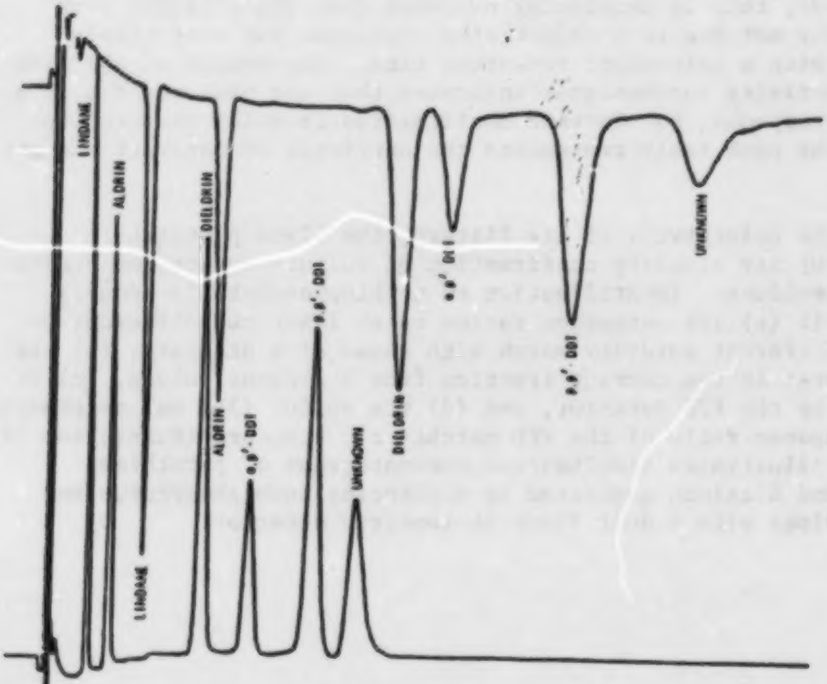
d. NEVER rely on one GC column for positive identification. Use an alternative column providing a completely different peak elution pattern.

As illustrated in Figure 5-A,A in Section 5, the combination of a nonpolar DC-200 column with a slightly polar DC-200/QF-1 column (plot A) is not very useful for confirmation. Another highly correlated pair of phases is slightly polar 4% SE-30/6% QF-1 with slightly polar 1.5% OV-17/1.95% QF-1. To the contrary, a combination of DC-200 with highly polar DEGS (plot B) or highly polar OV-210 with OV-17/QF-1 (plot C) would be a good choice. Other complimentary pairs are SE-30/QF-1 with either DEGS or OV-210.

Specific examples (5) of the utility of at least two different GC columns for sample diagnosis include the following. Identity of certain early eluting BHC isomers, particularly the alpha isomer, may be hindered by the presence of hexachlorobenzene. The latter is co-eluted with α-BHC on silicone columns and with β-BHC on Apiezon, but all three compounds are resolved on a polar cyano-silicone column. Dieldrin and *p,p*'-DDE are difficult to resolve on a number of single phase silicone columns but are separated on Apiezon, cyano-silicone, and trifluoropropyl silicone (QF-1, OV-210, SP-2401). On Apiezon, dieldrin elutes before DDE while the order is reversed on the cyano-silicone column. On the QF-1 or OV-210

column, dieldrin elutes far later than p,p'-DDE, to the extent of about 1.4x at 180°C column temperature. Figure 10-B illustrates the confirmation of organochlorine pesticides by comparison of relative retention times on two columns of different polarities.

Figure 10-B. Dual column confirmation of pesticides by electron capture detection. Pesticides (from left): lindane, aldrin, dieldrin, o,p'-DDT, p,p'-DDT, and unknown. Top chromatogram: 4% SE-30/6% OV-210 on Gas Chrom Q. Bottom chromatogram: 1.5% OV-17/1.95% OV-210 on Gas Chrom Q. Both columns: 6.3 mm x 183 cm glass. Carrier gas: nitrogen, 65 ml/min. Oven temperature: 200°C. Chart speed: 1.27 cm/min.



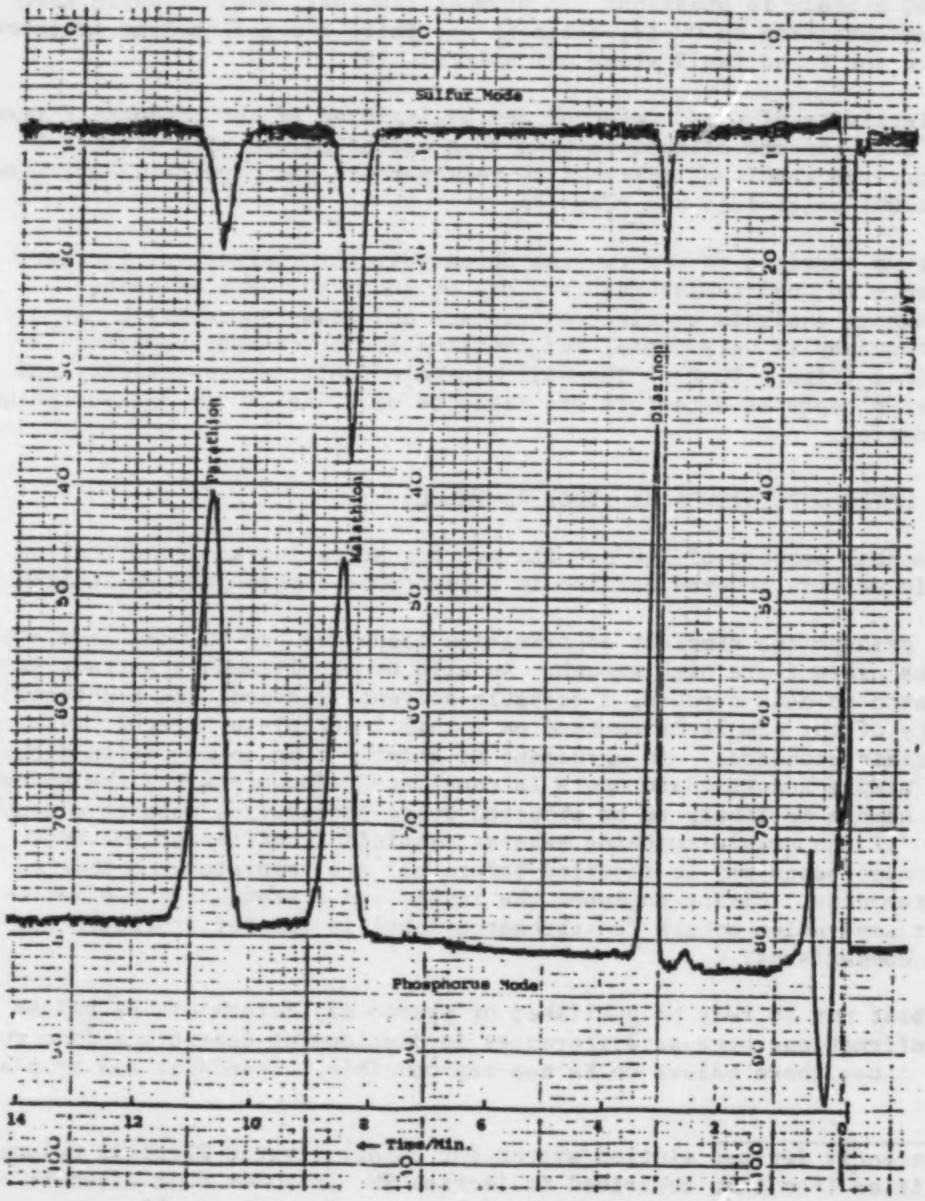
10C SELECTIVE GC DETECTORS

The EC detector, being rather non-specific, responds to any electron capturing compounds injected in addition to pesticides. For this reason, interpretation of results from EC-GC is facilitated if additional chromatograms are run using one or more of the highly selective detectors. The thermionic, flame photometric, or conductivity detectors, described in Subsections 5E, 5F, and 5G, are especially useful for confirmation. Because interference peaks may occur with even the most selective detectors available, the absence of a peak is really more conclusive than a positive response. For example, if a peak on an electron capture chromatogram suspected of being a chlorinated pesticide does not appear when the sample is injected into a chromatograph with the same column and a Hall conductivity detector in the Cl mode, this is convincing evidence that the original peak was definitely not due to a chlorinated pesticide but most likely an artifact with a coincident retention time. Appearance of the peak in the conductivity chromatogram indicates that the peak was due to a halogenated compound, but further confirmation is still required to prove that the peak truly represents the pesticide of interest and not an artifact.

Because of the selectivity of its filters, the flame photometric detector (FPD) may simplify confirmation of sulfur- and/or phosphorus-containing residues. Identification of a thiophosphate is usually unequivocal if (a) its retention ratios on at least two different GC columns of different polarity match with those of a standard, (b) the compound elutes in the correct fraction from a cleanup column, (c) it is detected by the FPD detector, and (d) the sulfur (394 nm) to phosphorus (526 nm) response ratio of the FPD matches the standard (Subsection 5F). Figure 10-C illustrates simultaneous chromatograms of parathion, malathion, and diazinon generated by monitoring both phosphorus and sulfur emissions with a dual flame photometric detector.

Figure 10C

Figure 10-C. Gas chromatograms of phosphorothioate pesticides obtained simultaneously with a dual flame photometric detector.



Relying on only one GC column may lead to incorrect identification, even with the added information from the Florisil column elution and the FPD. For example, phosalone and azinphosmethyl have the same relative retention time on an OV-1 GC column; both elute in the third (hexane-acetone, 85:15 v/v) fraction* from the 2% deactivated Florisil column recommended by the Canadian PAM (Section 9M, this Manual), and both respond equally to the FPD since each compound has one P and two S atoms per molecule. A second example is phosalone and phosmet (Imidan), both of which have the same retention on OV-17, elute in the third Florisil column fraction*, and have one P and two S atoms per molecule (6).

The selectivity of an EC detector can be improved if the products formed in the detector are allowed to pass to a second column with another EC detector; the resultant distinctive peak pattern can provide identification of OCl pesticides and PCBs (7).

When it is possible to use two gas chromatographic detectors, further confidence in qualitative accuracy can be achieved. For example, simultaneous analysis by electron capture and flame photometric gas chromatography is very useful for confirmation of organophosphorus pesticides (Figure 10-D). The Hall microelectrolytic conductivity and nitrogen-phosphorus detectors are likewise very useful for dual-detector confirmation.

10D THIN LAYER CHROMATOGRAPHY (TLC) R_F VALUES

Experimental aspects of TLC and its use for screening and quantitation of residues were covered earlier in Subsections J through M in Section 7.

TLC is perhaps the simplest confirmation technique for GC when levels of residues present are high enough. An aliquot of cleaned-up extract is evaporated to near dryness, a suitable solvent is added, and a detectable quantity of the sample is spotted on a thin layer plate together with appropriate standards. An agreement of about ± 2 mm in migration distance of the sample and standard spots is considered adequate since the movement of the sample is likely to be affected by co-extractives despite cleanup steps. If the sample contains several pesticides, different solvents and/or adsorbents may be required before all are separated and matched with standards. Mixing together the sample and a standard and observing whether separation occurs (co-chromatography) is another procedure for making comparisons.

It is best not to rely on published or previously determined R_F values for confirmations because differences in development conditions from run to run cause these values to be non-reproducible. Standards and samples

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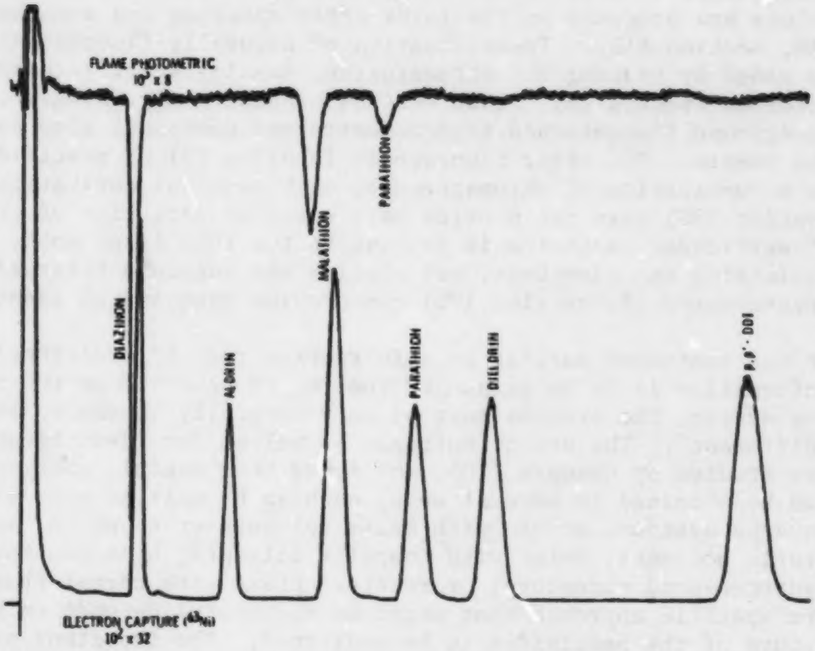
*/ A recently devised elution system for 2% deactivated Florisil columns, modified from that described in Section 9M, includes four eluents: hexane-methylene chloride (95:5 v/v), hexane-methylene chloride (70:30 v/v), hexane-acetone (85:15 v/v), and hexane-acetone (1:1 v/v (6).

Figure 10-D. Simultaneous gas chromatograms of organochlorine and organophosphorus pesticides using electron capture and flame photometric detectors. Columns: 6.3 mm x 183 cm, 4% SE-30/6% OV-210 on Gas Chrom Q. Carrier gas: nitrogen. Oven temperature: 200°C. Detectors: Pulsed ^{63}Ni, 270°C; flame photometric, 526 nm filter, 200°C. Chart speed: 1.27 cm/min.



should always be run on adjacent areas of the same plate if possible. If R_F values must be used, the value relative to the R_F of a standard compound X run on the same plate (R_X value) will be more reliable than the absolute R_F value for many of the same reasons that relative GC retention times are more reliable than absolute retentions. Chlorinated pesticides are often referred to DDD, and phosphates to parathion, in calculating R_X values.

Although TLC is very widely applied for pesticide confirmation, results may not always be conclusive. TLC confirmation of many pesticides, such as toxaphene and chlordane, is greatly influenced by the degree of cleanup on the sample extract and the level of detection. Oils and waxes will particularly interfere with TLC, causing streaked zones and/or distorted R_F values that may completely negate its value for confirmation. The 15% ethyl ether-petroleum ether Florisil column extract normally requires further cleanup prior to TLC (FDA Pesticide Analytical Manual, Section 411.5).

Detection reagents yielding spots of different colors with different pesticides are especially valuable for confirmation. Diphenylamine-zinc chloride reagent provides such differentiation for chlorinated pesticides; various shades of purple, grey, green, and reddish-orange colors are produced on the layer after spraying and oven heating (FDA PAM, Section 612). Identification of naturally fluorescent pesticides is aided by heating the chromatogram, causing specific alterations in recorded spectra (8). This heating procedure may, however, increase background fluorescence from co-extracted compounds also present in the sample. TLC after fluorogenic labeling (9) of pesticide residues is a combination of chromatography with chemical derivatization (Subsection 10G) that can provide very specific detection of certain residues. If sufficient pesticide is present in the thin layer spot, scraping, collecting the adsorbent, and eluting the compound followed by mass spectrometry (Subsection 10L) can provide unequivocal identification.

It was mentioned earlier in this section that if additional independent information is to be gained by running PC plus TLC or TLC in more than one system, the systems must be very carefully chosen to be truly "different". The use of multiple R_F values for identification purposes was studied by Connors (10), who found that useful, uncorrelated data can be obtained in several ways, such as by pairing aqueous with non-aqueous systems, acidic with basic solvents or supports, aprotic with protic solvents, polar with nonpolar solvents, hydrogen-bond donors with hydrogen-bond acceptors, or reversed phase with normal phase systems. The specific approach that might be successful depends on the chemical nature of the pesticides to be confirmed. The important point is that different thin layer and/or PC systems chosen at random will not necessarily provide the analyst with any additional, independent evidence of identity. Similar correlation studies were reported by Dale and Court (11).

Permanent records of TLC plates for documentation should be made by one or more of the following methods: Xeroxing the original plate, spraying with plastic to preserve the plate, hand tracing or charting, densitometry, or color photography (12). Where available, the latter appears to be the preferred procedure.

Section 614.11 of the FDA PAM describes a method for confirmation of organophosphorus pesticide residues by two-dimensional TLC. It is applicable at levels as low as 0.01 ppm in nonfatty food extracts cleaned up by carbon column chromatography. The pesticides are oxidized by bromine vapor after the first development, and detection is made with horse serum cholinesterase and indoxyl acetate substrate after the second development. The system provides good specificity because it involves chromatography of both the parent pesticides and their derivatives.

10E HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

See Subsections 7A through 7I in Section 7 for a discussion of this topic. HPLC has been used mainly for quantitation of residues in situations where GC is either not applicable or not convenient to use. An HPLC retention time can serve as evidence to confirm GC in the same way as a PC or TLC R_F value. The liquid chromatographic system should be carefully chosen to be "different" from the GC system (i.e., adsorption rather than partition), and the independence of the data must be clearly established if it is desired to use both PC or TLC and HPLC data for confirmation. The variable wavelength UV detector allows determination of the wavelength of maximum absorption for each pesticide. Detection of HPLC effluents with a Cl-selective electrolytic conductivity detector (13) can also provide useful confirmatory evidence.

10F EXTRACTION p-VALUES

Extraction p-values (14-18) are a tool for identifying pesticides at the low ng level. The p-value is determined by equilibration of a solute between volumes of two immiscible liquid phases, followed by the analysis of one of the phases for the solute. The p-value, defined as the fraction of total solute partitioning into the upper phase, can be derived from a single distribution between the solvents or from a multiple distribution, as in counter-current distribution. p-Values for most pesticides are appreciably different from those of normal co-extracted contaminants. The determination of these values is simplified since only relative, rather than absolute, data are required, and sensitivity is at or only slightly above the level of EC-GC.

Details including experimental procedures, formulas for calculating p-values and the fractional amount extracted after repeated extractions, graphs for determining specificity in a given system, and p-values for 131 pesticides in six binary solvent systems (hexane-90% DMSO, heptane-90% ethanol, isooctane-80% acetone, hexane-acetonitrile, isooctane-DMF, and isooctane-85% DMF) are given in Section 621 of the FDA PAM, reference (15), and Section 12,C of the EPA PAM (data for 88 pesticides in the latter). A device and method for determining p-values with unequilibrated solvents or unequal phase volumes are given in the FDA PAM, Section 622.1 and reference (18).

As mentioned earlier, the general technique of determining p-values has much in common with the use of several GC columns, PC, and TLC in identification studies since all systems may share the same partition mechanisms. Unless the analyst assures himself that the data are not correlated, it is best to use either a PC or TLC R_F value or an extraction p-value as one independent criterion of identity. The great advantage of p-values over PC or TLC is that the method is useful at levels amenable to quantitative analysis by EC-GC where sufficient residue might not be available for either of the former techniques.

10G DERIVATIZATION (CHEMICAL REACTION) TECHNIQUES

Derivatives of pesticides are prepared for various reasons, such as to decrease volatility or increase detectability for HPLC or TLC; to increase volatility, stability, and/or detectability and avoid tailing peaks for gas chromatography; for removal of interferences in residue analysis (19); and to alter the structure to aid characterization. It is this latter topic that will be discussed in this subsection.

Comparison of retention times on a given GC stationary phase before and after chemical derivatization is a relatively recent innovation that is becoming increasingly important for corroboration of residue identity. Desirable characteristics of any chemical derivatization technique include:

a. A specific product should be formed with at least as much or more response to electron capture, or to other detection, compared to the parent pesticide.

b. The product should have a different retention time than the parent, preferably greater to differentiate it clearly from the background.

c. Reactions should be essentially quantitative, they should use highly pure reagents and solvents, and they should be facile and rapid. Reagents and equipment should be inexpensive, if possible.

d. A cleanup method should be available to remove any background interferences introduced by the reaction.

e. If product structures and reaction mechanisms and limitations are known, misidentifications can be avoided because the analyst can elucidate the extent and probable sources of error in the procedure.

f. Sensitivity should be at least in the 0.01 to 0.1 ppm range in terms of the parent pesticide, which is lower than the established tolerance values for most pesticides.

g. The same reaction should occur, and to the same degree, in both the sample extract and in a solution of the reference material of the suspected compound at the same concentration. Matrix effects can play an important role in the applicability of chemical derivatization for quantitation purposes. A reaction might work very well for pure standards but may fail when applied to samples due to the effects of sample components.

h. The reaction should be safe to perform.

Derivatization reactions for gas chromatography are usually carried out in solution, on the surface of a solid matrix, or in a GC precolumn. Reactions in solution on a microscale are most common for residue level work. The reaction usually involves heating of the reactants in a small sealed tube, after which the derivative is dissolved in a suitable solvent. If direct injection into a gas chromatograph is not possible, cleanup by solvent partitioning and/or column chromatography and concentration steps may be applied. Solid matrix reactions are generally carried out by introduction of dissolved compound onto a microcolumn composed of solid support (e.g., alumina) mixed with reagent(s). After a specified reaction time, solvent is added to elute the derivative for GC determination. The advantages of this approach are simplicity, reduced glassware needs, and ability to react many samples simultaneously. However, the same derivative as formed in a solution reaction is not always produced and/or eluted from the column in a solid matrix reaction with the same active reagent. GC precolumns are usually composed of a reagent-solid support mixture located in a heated area ahead of the analytical column. The sample is injected into the precolumn, and the derivative is formed and swept by the carrier gas onto the analytical column for determination. Speed of operation is the greatest advantage for those reactions that are rapid enough to be feasible by the precolumn technique. The chromatograph is best fitted with a special injection apparatus so injection can be made into the precolumn for derivatization or directly into the analytical column for normal operation. In addition to these three types, some derivatizations may be carried out in a hot injection port or on the analytical column itself.

Derivatization aimed at increasing detectability in HPLC is usually carried out "post column", or after separation of the parent molecules rather than the derivatives. This is accomplished by inserting a mixing chamber at the end of the column and pumping in reagent to mix with the column effluent. The derivative is formed in a reaction coil, and is measured subsequently in a suitable detector. This type of derivatization can be easily automated for routine analysis. Derivatization for the purpose of providing detection in TLC is generally carried out by spraying chemical reagents, also "post chromatography". For both of these liquid chromatography techniques, derivatization for confirmation of identity is usually done in solution or "on column" (or "on-layer", for TLC), prior to chromatography, as is the case for GC.

The following subsections review some procedures for confirming residue identity by chemical derivatization. Table 651-A of the FDA PAM contains an extensive further listing of derivatization methods for more than 100 pesticides and related compounds of many chemical types, including comments on the level of applicability, yields, and 60 references to the original papers. A review paper on chemical derivatization for GC and HPLC has been published (20).

a. Organochlorine Pesticides

Most of the effort to date in the development of confirmatory derivatization tests has been confined to the organochlorine insecticides. For these compounds, addition, oxidation, epoxidation, rearrangement,

dechlorination, hydrolysis, reduction, and dehydrochlorination are the most commonly used reactions. Examples of specific tests are shown in Tables 10-1, 10-2, and 10-3, as reviewed through 1978 by Cochrane (20-22). Table 10-4 lists selected references for OCl pesticide derivatization methods published since 1978. Section 9A,G,e discusses confirmation reactions suitable for PCB-pesticide mixtures. It must be realized that these reactions destroy some pesticides (and artifacts) in addition to forming pesticide derivatives.

TABLE 10-1

CONFIRMATORY DERIVATIZATION TESTS FOR PESTICIDE AND METABOLITE RESIDUES (21)

Pesticide	**Reaction**
DDT	**(a) Dehydrochlorination** **(b) Dechlorination (of *p,p'*-isomer)**
DDE	**Oxidation**
DDD	**Dehydrochlorination**
Methoxychlor	**Dehydrochlorination**
Aldrin	**(a) Addition** Cl_2 Br_2 ***tert*-BuOCl** **(b) Epoxidation**
Dieldrin	**Epoxide** **cleavage** **rearrangement** **acetylation**
Endrin	**(a) Epoxide rearrangement** **(b) Dechlorination**
Endosulfan	**Sulfite reduction**
Heptachlor	**(a) Allylic** **acetylation** **hydroxylation** **dechlorination** **(b) Addition** **(c) Epoxidation**
Heptachlor epoxide	**Epoxide rearrangement**
***cis*- and *trans*-Chlordane**	**Dehydrochlorination**
Nonachlor	**(a) Dechlorination** **(b) Dehydrochlorination**

TABLE 10-1 (Continued)

Parent Pesticide	Metabolite	Group Reacted	Derivative
Heptachlor	(a) Chlordene	(1) Allylic hydrogen	1-Bromochlordene
		(2) Double bond	Chlordene epoxide
	(b) 1-Hydroxychlordene	(1) Allylic hydrogen	Silyl ether
		(2) Double bond	Chloroacetate
			Epoxide
	(c) 1-Hydroxy-2,3-epoxychlordene	(1) Hydroxyl	Silyl ether
		(2) Epoxide	Trihydroxy chlordane
trans-Chlordane	2-Chlorochlordene	Double bond or *gem*-dichloro group	Epoxide or hexachloro
cis-Chlordane	3-Chlorochlordene		
cis- and *trans*-Chlordane	1,2-Dichlorochlordene epoxide	Chloro epoxide or *gem*-dichloro group	Chloroacetate or heptachloro
Endrin	Photo-endrin	*gem*-Dichloro	Pentachloro
Endosulfan	Endosulfan diol	Hydroxyl	Acetate or silyl ether

TABLE 10-2

CONFIRMATORY TESTS FOR ORGANOCHLORINE PESTICIDES (22)

Pesticide Class	Reagent or Reaction Type
General	$CrCl_2$ reduction (26)[1] KOH dehydrochlorination (46)[1]
Hexachlorobenzene (HCB)	Base/alcohol KOH hydrolysis/diazomethane
BHC isomers	NaOMe/MeOH or GC alkaline precolumn
Cyclodiene insecticides	Comparison of 8 methods (D) 10 various reactions (D) BCl_3/2-chloroethanol (D/E) UV irradiation (D/E/H) H_2SO_4 or 60% KOH (E/M) t-BuOK/t-BuOH or $CrCl_2$ (E/M) Acid or base-Al_2O_3 microcolumn (C/E/H/T/M) Base-catalyzed intramolecular cyclization Silylation/acetylation (T/M)
Mirex	UV dechlorination
Kepone	KOH/esterification $LiAlH_4/PCl_5$
PCBs	$SbCl_5$ perchlorination
Chlorobiphenyls and PCP	Acetylation and butylation
DDT	Reduction and/or oxidation

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[1] Figures in parenthesis indicate the number of pesticides studied, letters indicate the particular pesticide(s) confirmed
C = chlordanes, D = dieldrin, E = endrin, H = heptachlor,
T = Thiodane (endosulfan) and M = and metabolites

TABLE 10-3

CHEMICAL DERIVATIZATION OF ORGANOCHLORINE, PCB, PBB, AND RELATED COMPOUNDS[a] (20)

Pesticide/ Compound	Derivatisation Procedure	Substrate
PCB/OC	Ethanolic KOH	Fish and fish prod.
	Ethanolic KOH/ CrO_3	Fish, serum
	Photoisomerisation (OCs)	Environmental samples
	Photo-dechlorination (PCBs)	
	"MgO microreaction"	Fish
PCB/OC/chlorinated paraffins	Photolysis	Fish
PCBs	TiO_2 photodechlorination	Aqueous media
	Perchlorination	Fish
Hydroxylated PCBs	Silylation	—
PCB/Mirex	Photolysis	Human tissue and milk
Mirex	Photolysis	Urine, feces, eggs
	Hematin dechlorination	Model system
Kepone	Chlorination	Blood, oyster
Kelvan	Photolysis or oxidation	Potatoes
PBB	Photolysis	Feeds, dairy prod.
HCB	2-propanol/KOH	Adipox tissue, human milk
Endosulfan	Photolysis	—
	Acetylation	Soils
Heptachlor and Epoxide	Photolysis	Foods
	$NiCl_2/NaBH_4$	—
	Acetylation	Soils
Lindane	$NiCl_2/NaBH_4$	—
Toxaphene	Methanolic KOH	—
Polychloro naphthalenes	Photolysis	—
TCDD	Photolysis	Silica, soil

[a] PCB = polychlorinated biphenyl; PBB = polybrominated biphenyl; HCB = hexachlorobenzene; TCDD = tetrachlorodibenzodioxin; OC = organochlorines.

TABLE 10-4

CONFIRMATORY DERIVATIZATION REACTIONS FOR OCl PESTICIDES PUBLISHED SINCE 1978

Compounds Studied	Reagent (sensitivity)	Reference
Chlordane and mirex	Conc. H_2SO_4-fuming HNO_3 (1:1 v/v) to remove PCBs and other interferences	23
Mirex and PCBs (in fish)	Reduction of mirex with chromous chloride	24
OCl pesticides and PCBs (Harp seal tissue)	Dechlorination using sodium ethoxide	25
Chlorophenoxy acid herbicides	Pentafluorobenzyl bromide (10 ppb in urine)	26

A confirmatory technique related to chemical derivatization is ultraviolet degradation or photolysis (27, 28; Table 652-A of the FDA PAM). Degradation products arising from UV treatment of chlorinated insecticides and detected by EC-GC can provide identification of these pesticides (28) at 75-100 pg levels. Depending on the length of irradiation (often ca 10 minutes), all of the parent pesticide may not be degraded. Solvent and sample blanks should be run to prove if background is reacted as well. Isooctane is a good solvent because it is little affected by UV light.

Section 12,D,(1) of the EPA PAM gives details of a microscale alkali dehydrochlorination method for use in multiresidue analysis. This procedure produces derivatives for identity confirmation and provides supplemental cleanup for some troublesome extracts after Florisil chromatography. Section 651.12 of the FDA PAM describes the microscale alkali treatment method that is part of the AOAC official method for perthane. Table 651.1 lists the behavior of about 40 compounds under these reaction conditions. Alkali reactions carried out on a GC precolumn rather than in solution have proved advantageous in some instances (29). Section 5,A,(1),(b) of the EPA PAM describes the confirmation of HCB in fatty tissue by formation of the disubstituted ether derivative bis-isopropoxytetrachlorobenzene (30).

Section 11 of the Canadian PAM gives complete details for the following tests:

Pesticide(s)	Reagent
o,p'-DDT, p,p'-DDT, p,p'-TDE, methoxychlor	Sodium methylate
p,p'-DDT, endrin	Chromous chloride
Dieldrin, endrin	BCl_3 in 2-chloroethanol
Chlordane, heptachlor epoxide	K-tert butoxide/tert-butanol, silylation
Aldrin, heptachlor, p,p'-DDE	Chromic acid
Aldrin	m-Chloroperbenzoic acid
Endosulfan	Alcoholic KOH
Chlorophenoxy acid herbicides	n-Propanol
Captan	Resorcinol

A special two stage, mixed phase 180 cm column consisting of 165 cm of 4% OV-1/6% QF-1 and 15 cm of 3% OV-1/6% OV-225 at the injector end is recommended in the Canadian Manual for resolving HCB, BHC isomers, sulfur, and aldrin for confirmatory purposes, because they are not resolved on the 4% SE-30/6% QF-1 working column.

One method of differentiating PCBs from organochlorine pesticides is by treating the residues with a non-fuming HNO_3-H_2SO_4 mixture. Organochlorine pesticides are destroyed, whereas PCBs (and toxaphene) are unaffected. Various confirmation methods for PCBs are covered in Subsection 9A,G,e in Section 9 of this Manual, and perchlorination is described in Section 12,D,(2) of the EPA PAM.

b. Other Pesticide Classes

Residues of organophosphorus pesticides may be confirmed by alkaline hydrolysis followed by esterification of the resulting dialkyl phosphates to trialkyl phosphates (31). This procedure does not distinguish pesticides that produce the same hydrolysis product. According to McCully (32), the three most practical methods for confirmation of OP pesticides are oxidation to oxygen analogs (33), pentafluorobenzyl bromide derivatization of hydrolyzed phenols or thiophenols (34), and chromous chloride reduction (35). The sodium hypochlorite oxidation method has the widest applicability, but it suffers from low sensitivity, difficulty in analyzing the analog products, and inability to distinguish analogs originally present in samples. The $CrCl_2$ method is simple but applicable only to OP pesticides containing a nitro group. The pentafluorobenzyl bromide procedure is intermediate in scope. These and other reactions used to identify organophosphorus pesticides are listed in Table 10-5, along with information on triazine, carbamate, and urea pesticides. This table is from a review article (22) that gives the original references for these reactions. Table 10-6 contains a selection of more recent references. The pentafluorobenzyl bromide derivatization procedure for OP pesticides is being collaboratively evaluated on different substrates (36).

Triazine herbicides have been confirmed by silylation, methoxylation (in sodium methoxide-methanol), methylation (CH_3I-NaH), and hydrolysis-DNFB reactions (37, 38), and linuron has also been confirmed by alkylation (with alkyl halide - NaH) (37).

TABLE 10-5

CONFIRMATORY TESTS FOR ORGANOPHOSPHORUS, TRIAZINE, UREA, AND CARBAMATE COMPOUNDS (22)

Pesticide Class	Compound Type	Confirmatory Test
Organophosphates	a) General	Hydrolysis/methylation
	b) Phenol-generating compounds	Hydrolysis/PFB ether formation
	c) Aryl-NO_2 and Aryl-CN containing compounds	Reduction ($CrCl_2$, $PdCl_2$, Zn/HCl)
	d) P=S compounds	Oxidation (to P = O)
	e) -NH and -NH_2 containing compounds	i) Alkyation (NaH/MeI/DMSO) ii) Deamination/methylation
	f) OH compounds (diazinon metabolites)	Silylation or alkylation
	g) Crufomate	UV dechlorination
Triazines	a) Chloro-*s*-triazines	i) Alkylation ii) Silylation iii) Methoxylation iv) Hydrolysis/DNP formation
	b) Hydroxy-*s*-triazines	i) Silylation ii) Alkylation iii) Chlorination
	c) Cyanazine and metabolites	Acid catalyzed cyclization
Carbamates and ureas	a) Intact compound	i) Acetylation ii) Silylation iii) Alkylation iv) Perfluorination*
	b) Phenol-generating compounds	i) Bromination ii) Chloroacetylation iii) Thiophosphorylation iv) Silylation v) Dichlorobenzene sulfonylation vi) DNT/DNP vii) Pentafluorobenzylation
	c) Amine-generating compounds	i) Iodination ii) Bromination iii) *p*-Bromobenzoylation iv) 2,4-DNP v) DNT vi) Pentafluorobenzylation (Amines in general)
Chlorophenoxy acids	a) Esters	i) Transesterification ii) Bromination

*Includes trifluoroacetylation, pentafluoropropylation, and heptafluorobutylation

TABLE 10-6

CONFIRMATORY DERIVATIZATION REACTIONS FOR PESTICIDES OF VARIOUS CLASSES PUBLISHED SINCE 1975

Compounds Studied	Reagent or Derivative (Sensitivity)	Reference
$-NO_2$-containing herbicides and fungicides	$CrCl_3$ reduction to $-NH_2$ followed by CCD-GC (0.5-1.0 ppm)	(39)
Organonitrogen fungicides and herbicides	Alkylation, methoxylation, trifluoroalkylation (0.1 ppm)	(40)
Carbofuran and metabolites	Heptafluorobutyric anhydride plus trimethylamine catalyst (10 pg)	(41)
OP pesticides	In-block methylation with THAM (μg levels)	(42)
Sulfoxide-containing pesticides	Trifluoroacetic anhydride (1 ppm)	(43)
Carbaryl	N-mono- and trichloroacetyl, and N-nitroso derivatives	(44)
Carbamate insecticides	Heptafluorobutyryl derivatives (0.1 ppm)	(45)
Dimilin (TH 6040)	Trifluoroacetyl derivative (0.02 ppm)	(46)
Thiabendazole	Pentafluorobenzyl chloride (0.01 ppm)	(47)
N-Aryl carbamates	Flash heater reaction with trimethylanilinium hydroxide (ng levels)	(48)
S-Containing carbamates	Trimethylphenylammonium hydroxide injected with compound into gas chromatograph (20 ng)	(49)
Carbamate and urea herbicides	Alkylation by NaH/CH_3I (0.1 ppm)	(50)
Dimilin (TH 6040)	Conversion to N,N'-dimethyl analog with NaH/CH_3I (0.25 ng)	(51)
Azodrin	Trifluoroacetylation (2 ppb)	(52)
OP pesticides	Oxidation with neutralized NaOCl (0.25-0.5 ppm in fruits and vegetables)	(53)
s-Triazines	Trifluoroacetic acid plus a silylation reagent (20 pmol)	(54)
N-Methyl carbamate insecticides and metabolites	Post-column HPLC fluorometric labeling with o-phthalaldehyde after basic hydrolysis	(55)

10H SPECTROMETRY (SPECTROPHOTOMETRY)

Spectrophotometric methods for residue determination (quantitation) usually are not as sensitive or selective as GC or TLC, and for this reason they are not as widely used as in the early days of pesticide analysis before chromatographic methods were developed. The applicability of spectrometry is especially limited for multiresidue determinations or analyses of a parent compound, metabolites, and hydrolysis products.

Spectrometry can be very valuable, however, in conjunction with chromatography as a confirmatory tool, and it is this aspect that will be stressed in the following subsections.

10I VISIBLE, UV, FLUORESCENCE, AND PHOSPHORESCENCE

Very few pesticides are naturally colored, so a chromophoric group must be formed by a reaction or added through derivatization before most pesticides can be measured in the visible spectral region. The colorimetric method then becomes specific to the color forming group involved. The inferior sensitivity of direct and indirect visible spectrophotometric methods limits their usefulness for confirmation in human and environmental monitoring where residues are generally present at low concentrations.

The correlation between UV spectra and pesticide structure and the usefulness of UV spectrophotometry in confirming identification have been reviewed (56). Spectra-structure correlations can be of value to the analyst in identifying chromophores and therefore making confirmations, especially in conjunction with spectral information obtained by other methods, such as IR, NMR, and MS. In some cases, extinction coefficients (absorptivities) are sufficiently large to permit identifications at submicrogram levels. If a suitable absorption wavelength of a pesticide can be chosen that is free of interference from contaminants or solvents, UV spectrophotometry can be performed directly without sample purification and at a greater saving of time. However, because absorption of UV energy is quite common for most organic compounds, rigorous cleanup may be required to remove any interferences that can absorb in the spectral region where the pesticide will be measured. The transparency of many functional groups (and often large segments of complex molecules) in the near UV spectral range imposes a limitation on interpretations of absorption bands in this region. Solvents must be carefully chosen to be transparent at the wavelengths absorbed by the pesticide. UV absorbing groups can be added by chemical derivatization methods, and this procedure has been used to detect pesticides by HPLC UV detectors and by TLC. UV spectra of 76 reference pesticides have been published (57). Visible and UV spectrophotometric methods for pesticides have been reviewed (58), including development of color by azo coupling and π complexing and recent instrumental developments.

If a pesticide is naturally fluorescent or can be made fluorescent by derivatization, fluorescence spectrophotometry is likely to be more selective and sensitive than either visible or UV absorption methods. Concurrence of fluorescence excitation and emission spectra between samples and standards, recorded either in solution or directly on thin layer chromatograms, has served as a valuable confirmatory aid for certain pesticides. Fluorescence characteristics are dependent on a number of experimental conditions which must be closely controlled, e.g., solvent and pH effects (59). Removal of naturally occurring fluorescent interferences from biological samples can pose serious cleanup problems. Fluorescence and phosphorescence methods for pesticides have been reviewed by Argauer (60), and the phosphorimetry of pesticides has been reviewed by Baeyens (61).

10J INFRARED (IR)

IR spectroscopy with micro sampling techniques is generally sensitive at the 1 μg level but has been used as low as the 0.1 μg level in some applications. It is thus considerably less sensitive than GC or TLC and cannot be used unless enough sample is available to provide a sufficient concentration of pesticides for IR observation. Sample extracts require a stringent cleanup procedure (e.g., partition plus column adsorption chromatography) plus additional purification either by GC or TLC. Thin layer spots are scraped and collected, and the pesticide is eluted from the adsorbent with an appropriate solution. Fractions can be collected from a gas chromatograph equipped with a stream splitter: a small percentage of the effluent stream goes to the detector for monitoring purposes while the remainder goes to a collecting device.

Potassium bromide (KBr) micro-pellet techniques using a pellet of 1-2 mm diameter are described in Section 12,E of the EPA PAM. These methods were developed by R. C. Blinn of the American Cyanamid Co. The key to their sensitivity is the ability to transfer the maximum amount of pesticide to a very small amount of KBr to be pressed into the micro-pellet. The equipment commonly used by Blinn for preparing micro-pellets by the syringe method is shown in Figure 10-E, and the technique for transfer of the sample-KBr mixture adhering to the syringe needle is pictured in Figure 10-F. The method using a commercially available "wick stick" may be the most reliable and foolproof for preparing micro KBr pellets (Figure 10-G). The sample is applied to the wedge of potassium bromide, which is then dipped at its base into a volatile solvent. The solution migrates up the wedge to the tip where the solvent evaporates, and the compound becomes concentrated at the tip. The tip is then cut or broken from the wedge and pressed into a micro-pellet. A procedure using micro pellets of 0.5 mm diameter to measure 1.4 μg of DDT has been reported (62).

The Blinn microtechniques are sensitive and reliable, but considerable experience is required to prepare pellets with a minimum of contamination. They require the availability of a modern IR spectrophotometer including a beam condenser and microcells. Contamination from such sources as the sample, solvent, reagents, atmosphere, and handling is their major source of error. The same amounts of interferences that would be inconsequential for macro-sampling techniques become a significant percentage of a micro sample and contribute to the spectrum. Clean gloves should always be worn when preparing micro-pellets, and only purified solvents and reagents and carefully cleaned equipment should be used. Inevitable losses due to handling and processing require that the isolation procedure be started with sufficient sample to finally achieve a useable spectrum.

Another method that is in effect in a micro-sampling technique involves scale expansion, or electronic amplification of the signal from the spectrometer. This method increases pen response without an increase in the sample concentration, but the response to all interferences and electronic noise is increased as well. All sources of interference must, therefore, again be minimized.

IR microtechniques have been reviewed by Blinn (63), including discussion of micro multiple internal reflectance. Advantages of internal reflectance include ease of applying (by dotting or streaking) sample to the surface of the reflectance plate (crystal), minimizing of interferences from handling and reagents, and ease of recovery after IR evaluation (samples made into pellets are essentially lost for further scrutiny). A disadvantage is lowered sensitivity compared to the KBr micro-pellet method. Sensitivity is increased by spreading a very thin film of sample over the effective sample area of a very thin reflectance plate. The multiple reflectance method has been applied to the identification of Thiram residues at 0.1 ppm on lettuce after extraction, Florisil chromatography, and TLC (64). An alternative micro-KBr technique with sensitivity levels similar to the method in the EPA PAM is detailed in the FDA PAM, Section 631.

Figure 10-E. Equipment for preparation of micro KBr pellets (photo courtesy of R. C. Blinn)

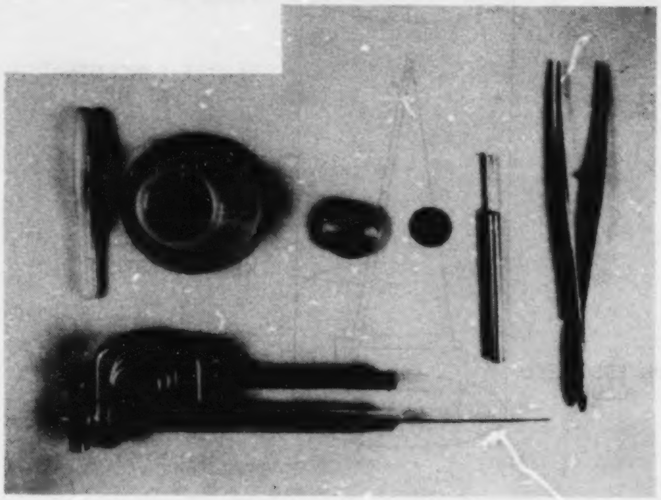
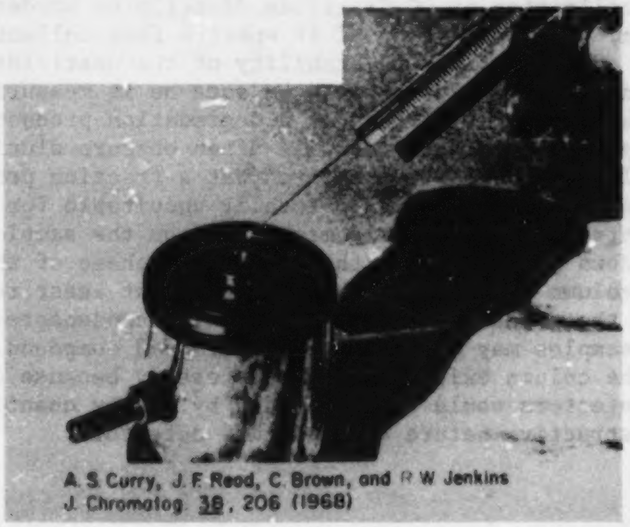
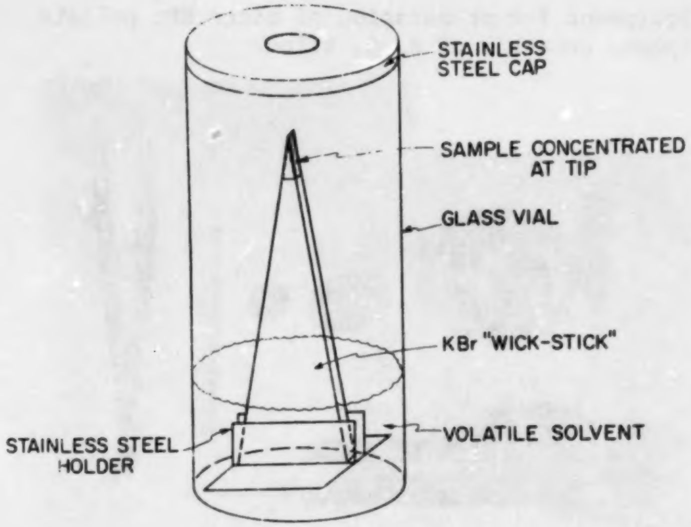


Figure 10-F. Illustration of technique of Curry et al. (Photo courtesy of R. C. Blinn)



A S Curry, J F Reed, C Brown, and R W Jenkins
J. Chromatog. 38, 206 (1968)

Figure 10-G. Illustration of wick-stick method



The FDA Manual (Section 632) also gives details of a qualitative micro procedure for collection of GC fractions directly on powdered KBr for IR confirmation. Interpretation of IR spectra from collected fractions must take into consideration the stability of the pesticide of interest to GC conditions. The analyst should be sure he is measuring the spectrum of unchanged pesticide rather than of a degradation product. In addition, the specificity of the GC detector will often obscure elution of interfering materials from the GC column, so that a fraction presumably containing isolated pesticide could be totally unsuitable for IR characterization. These interfering materials might be from the sample substrate or bleed or breakdown products from the stationary phase of the column packing. The column exit line should be heated at least to column temperature to the point of trapping, otherwise condensates resulting from previous samples may contaminate the trapped compound. Use of splitters at the column exit is usually necessary because of the high sensitivity (detectors would be overloaded by the μg quantities for IR) and the destructive nature of pesticide detectors.

Several different types of IR detectors directly coupled with gas chromatographs (65) have become available commercially but have not proven especially useful for pesticide residue work because of various disadvantages. Trapping procedures have been used almost exclusively, including the following methods:

a. Passing column effluent through solvent (66, 67).

b. Condensing effluent on a micro sodium chloride plate.

c. Condensing effluent on a thermo-electrically cooled capillary plate for internal multiple reflectance IR.

d. Trapping fractions on column packing (68-70). This procedure is very efficient, and fractions are easily collected for subsequent IR evaluation; reagent interferences are possible.

e. Collecting on a TLC plate for further cleanup prior to IR (71).

f. Trapping on Millipore or siliconized filter material (72, 73).

g. Using various types of liquid nitrogen or dry ice cold surface traps (74).

h. Using a cool or cold small internal diameter tubing at the GC vent (75).

i. Trapping directly on KBr powder supported by pipe cleaner inside capillary tubing (FDA PAM, Section 632). This procedure is probably the most sensitive of any, tubes can be changed for each peak, and the technique is free of sources of interferences.

The choice of trapping procedure will depend on the amount of compound available, IR technique to be used, purity of the compound eluted from the GC column, and equipment available to the chemist.

IR spectra of over 400 reference pesticides have been published (76) to aid the analyst in matching spectra of unknown pesticides. The ASTM FIRST-1 computer search program (65) and similar computer retrieval systems aid in matching sample and reference spectra when standards cannot be easily chosen for a manual point-by-point comparison. Reference Raman spectra of OCl, OP, and carbamate pesticides were published (77). Giang has compiled a bibliography with 855 references published to 1976 on the use of IR spectrophotometry in pesticide analysis (78).

An important recent development in IR analysis that is capable of sensitivity at subnanogram (79) residue levels is the Fourier transform (FT) or interferometric method. In FT-IR, a Michelson interferometer is used instead of the prism or grating and slits in a conventional spectrometer. The slitless spectrometer has an advantage in energy throughput, in addition to the so-called Fellget's or multiplex advantage that allows all wavelengths to be detected simultaneously throughout the spectral range. The signal to noise ratio increases with consecutive accumulation of scans and is proportional to the square root of the number of scans. Since each scan requires only a few seconds and instrument stability is high, many cumulative scans can be made on each sample. The fast scan capability is ideal for on-the-fly IR detection of GC effluents. FT-IR spectroscopy has at least an order of magnitude greater resolving power, greater wavelength accuracy, and a greater scan range than does conventional dispersion IR spectroscopy. There is also a much smaller image in the sample compartment without any special measures, making FT-IR ideal for microsamples. A dedicated minicomputer, in addition to the basic FT-IR optical equipment and detector, is required to collect, process, and store the data. FT-IR methodology and equipment have been reviewed (65). There is no doubt that much use will be made of FT-IR spectroscopy for pesticide determination and confirmation as the principles, techniques, and instrumentation become more familiar.

10K NUCLEAR MAGNETIC RESONANCE (NMR)

NMR spectroscopy has had only limited application in residue analysis because of its low sensitivity relative to other analytical methods, e.g., GC-MS, IR, and UV. Despite this drawback, it is one of the most valuable tools available for structural analysis and identity confirmation. Current pulsed Fourier transform NMR spectrometers (80) allow routine acquisition of useful data on as little as 10 µg of a proton NMR sample in a few minutes of experimental time. The NMR sensitivity of ^{13}C is lower; with current commercial instrumentation, a practical sample size is greater than 20 mg, although ^{13}C spectra of as little as 300 µg have been obtained on modified instruments (81). Useful information is provided by NMR in many areas relevant to the analysis of pesticides, their metabolites, and degradation products, such as identification and structural characterization, molecular geometries, conformations and stereochemistry, chemical kinetics and equilibria, complex formation and binding, and electronic charge distributions.

Residues of p,p'-DDT and p,p'-DDE isolated from adipose and liver tissue samples have been analyzed by NMR (82), with semi-quantitative determination of the relative concentrations of the pesticides. Included in NMR studies of the metabolism, binding, and degradation of pesticides are ^1H spectra useful for identification of p,p'-DDT (83, 84), p,p'-DDA (85), aldrin and dieldrin derivatives and other chlorinated pesticides (86), rotenoids (87), and dithiocarbamates (88). Other ^1H reference spectra of organophosphorus (89), diphenylmethane (DDT type) (90), and carbamate (91) pesticides have been published and are useful for identity confirmation. The application of NMR to pesticide analysis has been reviewed (80, 92, 93).

Carbon-13 NMR spectra have been published for α-BHC (94), for several chlorinated biphenyls (95, 96), and for 30 chlorinated polycyclodiene pesticides (97). Studies of technical chlordane components (98), mirex (99, 100), and Kepone and its photo-products (101) have provided ^{13}C

NMR data useful for confirmation and structural characterization. Chlorine nuclear quadrupole resonance spectrometry has been used to study the structures of several chlorinated pesticides including BHC, aldrin, endrin, endosulfan, and dieldrin (102-104). ^{31}P-NMR chemical shifts have been correlated with structures of some organophosphorus pesticides (105), and ^{31}P Fourier transform NMR has been used for the determination of malathion at ppm levels (106).

10L MASS SPECTROMETRY (MS)

The mass spectrometer is a very sensitive spectroscopic tool for pesticide residue analysis, providing useful data on ng or pg residue levels. Ions are produced from neutral sample molecules and are then sorted according to their mass-to-charge ratio (m/z). The mass spectrum is a record of these different ions and their relative abundance. A mass spectrum is usually quite characteristic of an individual pesticide, sometimes even providing data that will differentiate geometric isomers. Pesticide identifications can be made by matching the mass spectrum of an unknown sample with the mass spectrum of a known material. This comparison is especially valuable because it is based on many different peaks characteristic of the unknown compound. The composition of an unknown compound can be obtained without comparison to a reference material by making exact mass measurements of the molecular ion and other key fragment ions in the spectrum. Because the exact mass of every element has a unique fractional value on a scale compared to $^{12}C = 12.0000$, any combination of these elements into a chemical formula will have a unique fractional mass, specific for that combination of elements. The exact masses used to determine the chemical composition of a compound can be obtained on either a low or high resolution spectrometer. The advantage of a high resolution instrument is the ability to separate ions with different compositions that are at the same nominal mass (e.g., m/z 28 for CO, N_2, C_2H_4) and to obtain accurate mass values for these ions. The molecular ion is the species resulting from the removal of a single electron from a molecule. After the recommendation of Benyon (107), the symbol M   is used to represent the odd-electron molecular ion formed from an even electron molecule.

a. MS Instrumentation and Operation

(1) Introduction

Five components are common to most mass spectrometers: the inlet system, the ion source, the mass analyzer, the detector, and the readout system. In addition, a vacuum must be maintained throughout the spectrometer from inlet to detector so that ions formed in the source will not be lost from collisions with atomspheric gas molecules. A second reason for maintenance of vacuum is to prevent oxidation of the filament in the ion source and various other inside parts of the spectrometer and electron multiplier. A sample is introduced via the inlet system into the ion source, where it is ionized. The function of the inlet system is to transfer the sample from a high pressure (i.e., 1 atm) region into the vacuum of the spectrometer without seriously unbalancing the spectrometer operation. The generated beam of ions is focused and separated in the mass analyzer according to the m/z ratios. The detection system senses the mass-separated ion beams, and the readout device translates the signal provided by the detection system into an output that can be interpreted by the analyst. Several reviews of pesticide residue analyses by MS have been written by Safe (108), Skinner and Greenhalgh (109), and Ryan (110). In addition, detailed reviews of mass spectrometry have been made by Burlingame et al. (111) and Alford (112).

(2) Inlet Systems: Direct Insertion Probe

Samples may be introduced directly into the ion source with a direct insertion probe assembly. For example, the sample is loaded into a short length of melting point capillary, placed in the heater well at the end of a probe, and inserted to within a few millimeters of the ion source through a vacuum lock that maintains a vacuum-tight arrangement. The temperature is then increased until the sample vaporizes and a spectrum is obtained. The introduction of trapped GC fractions into an independent mass spectrometer by these techniques was used for residue analysis prior to the development of combined gas chromatography/mass spectrometry (e.g., 113), but the latter procedure is now employed almost exclusively. The direct insertion probe is reserved mainly for samples that cannot be chromatographed, because of thermal instability, low vapor pressure, and/or high polarity. However, combined with specific ionization techniques, such as negative chemical ionization, direct insertion can provide a sensitive, rapid screening method (113A).

Combined GC/MS

For impure samples such as biological extracts, the gas chromatograph of a coupled GC/MS instrument serves as an efficient inlet system for introduction of samples into the spectrometer. The resolution provided by gas chromatography offers extra sample cleanup in addition to any partition and liquid chromatography steps. Temperature and sometimes flow rate programming have proven useful for achieving high chromatography resolution with the combined instrument. Column bleed can be a serious proble in GC/MS, since bleeding liquid phase is also detected by the mass spectrometer and contributes spurious ions to the analytical spectra. Carefully conditioned, low bleed columns that are stable at high temperatures should be used whenever possible. Other approaches that alleviate problems from column bleed include use of a short, bleed-absorbing column placed between the analytical column and the GC/MS interface, programming the flow rate of the carrier gas, and computer subtraction of background resulting from the bleed (114).

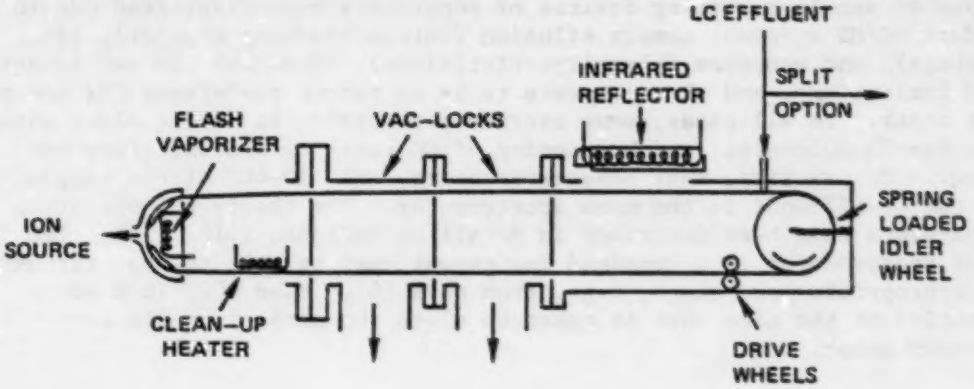
Compatability of the gas chromatograph and mass spectrometer is a problem because of the large volume of carrier gas eluting from the chromatograph and the need to operate the spectrometer at high vacuum (10^{-5} - 10^{-6} mm Hg). In the simplest approach, the two instruments are connected directly, and a large pumping system is used to maintain the required vacuum in the mass spectrometer. This approach has been used successfully with GC columns having flow rates up to ca 5.0 ml/minute. Introduction of samples from packed columns into the mass spectrometer requires removal of most of the carrier gas by means of an interface between the two instruments. At the same time, as much sample as possible should be retained so that the gas flowing into the spectrometer is enriched in sample. Three basic types of sample enriching devices or separators have widespread use in modern GC/MS systems, namely effusion (Watson-Biemann; Brunnee), jet (Ryhage), and membrane (Llewellyn-Littlejohn). Each has its own advantages and limitations, and there appears to be no strong preference for one over the other. In all cases, some carrier gas enters the source along with the sample molecules, and broadening of GC peaks by the interface may occur. In practice, most separators convey only 20-40% of the sample in the GC effluent to the mass spectrometer. The theory and operation of separators have been described in detail by McFadden (115, 116). The mass spectrometer in a combined instrument must be able to scan through an appropriate mass range, e.g., from mass 10 to mass 800, in a small fraction of the time that it takes to elute the peaks from the gas chromatograph.

Combined LC/MS

GC/MS is sometimes limited by the volatility or heat sensitivity of the compounds under study. To circumvent these difficulties, various methods of interfacing a high pressure liquid chromatograph with a mass spectrometer have been explored. The demands on a LC/MS interface are much more extreme than for GC/MS, because of the greater enrichment required (usually 10^5) and the possible adverse effects (e.g., background interference, chemical ionization effects, filament damage, etc.) of excess solvent entering the ion chamber. Six methods have been used for LC/MS interfacing. Three methods, namely the high capacity atmospheric pressure ionization source, the semipermeable dimethyl silicone membrane, and modification of sample at the interface by reduction to hydrocarbon, have not been widely accepted. Methods involving direct introduction with no enrichment, direct introduction with jet enrichment, and mechanical transfer using a moving wire with belt are most promising and are under active development. All six methods have been described and compared by McFadden (116), with appropriate original literature references.

The most common commercial liquid chromatograph/mass spectrometer interface (117) (Figure 10-H) consists of a continuous belt that introduces the LC effluent into a chamber at atmospheric pressure and then sequentially passes it beneath an infrared heater and through two vacuum locks into a vaporization chamber. Under optimum conditions the LC solvent is evaporated from the belt by the heater and vacuum locks, leaving only a deposit of the sample on the belt. The vacuum locks also accomplish the transition from atmospheric pressure to the vacuum system of the mass spectrometer. In the vaporization chamber, a second heater volatilizes the sample in front of a nipple leading into the ion source. A third heater cleans the belt before its return to the atmospheric chamber via the vacuum locks.

Figure 10-H. HPLC/MS interface developed under contract by Finnigan Corporation* for EPA.


LC EFFLUENT
INFRARED REFLECTOR
SPLIT OPTION
FLASH VAPORIZER
VAC–LOCKS
ION SOURCE
SPRING LOADED IDLER WHEEL
CLEAN–UP HEATER
DRIVE WHEELS

This interface is able to accommodate most commonly used organic LC solvents at optimum flow rates varying from about 0.2 to 1.5 ml/minute. The use of water as an LC solvent generally requires an LC effluent splitter if reasonable LC flow rates are to be used, since the maximum capacity of the interface for water appears to be about 0.1 ml/minute. The LC/MS system has been successfully applied to the analysis of a large number of carbamate pesticides (117).

The field of LC/MS is still under development. A second LC/MS interface has been introduced by Hewlett-Packard, and other commercial interfaces are anticipated in the near future.

(3) Ionization Processes: Electron Impact (EI)

The most widely used ionization source is the electron impact type wherein gaseous molecules are ionized by electrons emitted from a from atmospheric pressure to the vacuum system of the mass spectrometer. In the vaporization chamber, a second heater volatilizes the sample in front of a nipple leading into the ion source. A third heater cleans the belt before its return to the atmospheric chamber via the vacuum locks.

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* Mention of commercial products does not constitute endorsement by the U.S. EPA.

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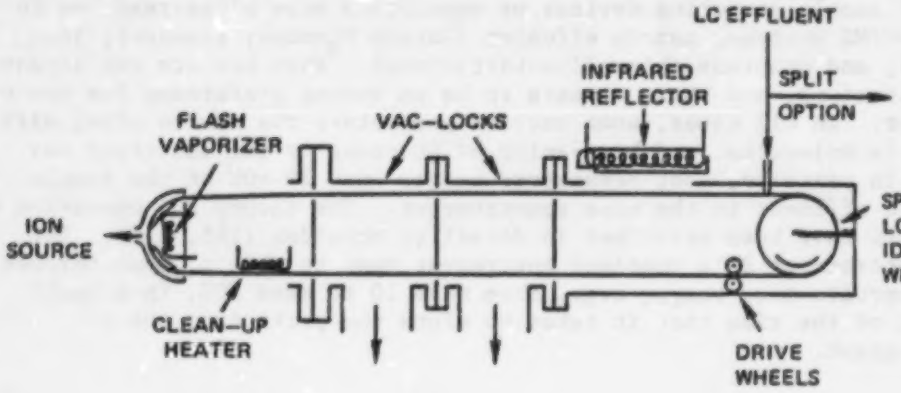

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(3) Ionization Processes: Electron Impact (EI)

The most widely used ionization source is the electron impact type wherein gaseous molecules are ionized by electrons emitted from a glowing filament. These positive ions are accelerated into the analyzer section. The EI source is relatively stable and easy to operate, and has high ionization efficiency. The overall quantity of positive ions and the nature of the fragmentation process depend on the energy of the ionizing electron beam.

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* Mention of commercial products does not constitute endorsement by the U.S. EPA.

Upon ionization of many compounds at low electron energy levels (0-20 electron volts or eV), a large fraction of the ion current tends to be carried by unfragmented molecular ions. However, the absolute intensity is relatively low. At higher energy levels, fragmentation and rearrangement are more prevalent, and the ion current is much higher. Molecules are often cleaved to such an extent that the molecular ion is absent from the mass spectrum or is of very low intensity. (A great many other compounds form only fragments even at low eV values.) Because mass spectra are more reproducible when compounds are ionized by 60-80 eV electrons, most mass spectrometers are operated in this energy range. It is noteworthy that the EI source produces mass spectra that are quite repeatable among instruments and distinctively characteristic of the compounds being ionized. This has led to the collection of large libraries of mass spectral data with which unknown spectra can be compared. Such comparisons often permit rapid identification of the unknown pesticide.

Chemical Ionization (CI)

Chemical ionization spectra are obtained by adding methane, helium, or other reagent gas (at relatively high pressures of about 1 mm or 130 Pa Hg) to the sample either as the GC carrier gas or after removal of the GC carrier gas by the separator. In the latter case, the CI reagent gas is introduced into the mass spectrometer just ahead of the point at which the effluent enters the ion source, or into the source itself. Electrons produce reagent gas ions that subsequently ionize sample molecules by chemical reactions, e.g., proton transfer, hydride abstraction, ion attachment, and resonance transfer. The mass spectra obtained with CI are quite different from those formed on electron impact and are, in general, simple and complementary to electron impact spectra for pesticide confirmation. Although CI usually provides molecular ion ($M^{+\cdot}$) or $(M + H)^+$ or $(M - H)^+$ peaks of high intensity, a study (118) of a series of chlorinated and organophosphorus pesticides found no molecular ion or ions in the molecular ion region produced from electron impact or chemical ionization for a number of specific compounds. CIMS has sensitivity at least as good as that of EI (119) and offers the advantage of allowing characterization of a sample's chemical reactivity through the choice of the reagent gases. In addition to methane and helium, other gases including isobutane, hydrogen, argon-water, ammonia, and nitric acid have been used successfully to produce CI spectra (120). Positive CI data for 29 OP insecticides and metabolites have been reported (121).

Field Ionization (FI)

Field ionization involves passing a gaseous compound between an anode (usually a thin wire or sharp blade) and a cathode. An extraordinarily high electric field, approximately 10^8 V/cm, is impressed on the anode, permitting (as most commonly explained) valence electrons of the sample to "tunnel" to the metal of the wire or blade. Charge separation can take the form of electrons tunneling out of molecules, proton transfers (facilitated by tuneling) between molecules, separation of the oppositely charged ions in electrolytes, and so on (111). A positive ion results, which can be separated according to mass-to-charge ratio and detected. This is a relatively low energy ionization method that often produces enhanced molecular ion intensities and a cleaner spectrum for compounds with poor thermal stability.

Fragmentations are less prevalent and different from those observed in normal EI spectra. The FIMS of a number of pesticides has been studied (122), and FIMS has been combined sequentially with HPLC for the determination of trifluralin (123).

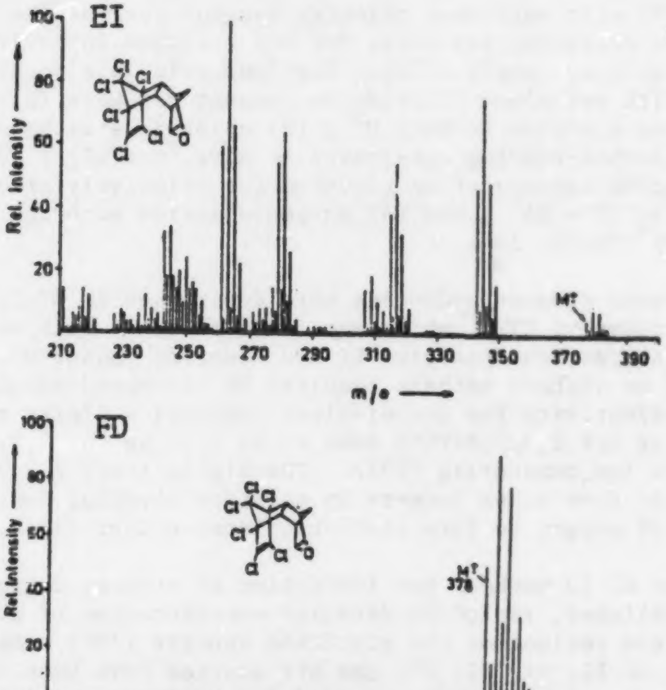
Field Desorption (FD)

Field desorption MS is a modification of FI in which the sample is applied directly to a carbon or metallic filament anode. As with FIMS, field desorption depends on application of very high electric fields (5000-10,000 V) to this anode. Sample molecules in contact with the anode desorb as ions into the source, where they are separated and mass analyzed. Like FI, field desorption produces ions of low internal energy, and usually results in minimal sample fragmentation. Unlike FI, field desorption has no requirement that the compound be volatile prior to ionization. Mass spectra can be obtained for samples that are thermally unstable or have no appreciable vapor pressure, as for example, salts. The field desorption mass spectrum of endrin and its EI spectrum, which has a low abundance of the molecular ion, are shown in Figure 10-I (124). Strong molecular ion peaks are produced for most pesticides (118), including highly polar pesticides and metabolites such as carbamates and ureas (124-126). Impurities may also give only molecular ions, so interpretation of mass spectra is sometimes simplified and the necessity of sample cleanup reduced. However, assignment of molecular ions and interpretation of spectra in biological samples can be complicated by the presence of $(M + H)^+$, $(M + Na)^+$, or other ion adducts and cluster ions. Other disadvantages are that quantitative data are difficult to obtain by FDMS, and valuable structural information provided by fragmentation is lost.

Atmospheric Pressure Ionization (API)

A novel method with high sensitivity (10^{-12} - 10^{-15} g) involves generation of ions with an atmospheric pressure ionization source. The API instrument is essentially an electron capture detector with suitable interfacing to a mass spectrometer so that its ions can be mass-identified. The source uses ^{63}Ni on gold foil to produce electrons that can interact with nitrogen and water passing through the ionization chamber at atmospheric pressure. Preheated carrier gas enters the API source just behind the sample injection port. Both gas and sample pass through the ^{63}Ni source block where ionization reactions take place. Just beyond the chamber is a small aperture through which the ions pass on their way to being mass analyzed and detected. With certain samples, this source generates more ions for a given quantity of sample molecules than any other ion source; this is reflected in the reference to the API mass spectrometer as the "femtogram machine" (127). The ^{63}Ni source has been replaced by a corona discharge (128), producing identical API mass spectra and limits of detection but a greater dynamic response range. Qualitative and quantitative applications of negative ion formation from pesticides in the API mass spectrometer have been studied (129).

Figure 10-I. Electron impact (EI) and field desorption (FD) mass spectra of endrin (124).



Negative Chemical Ionization

An important new development in chemical ionization methodology is simultaneously pulsed positive and negative CI mass spectrometry, developed by Hunt et al. (130). In this method, both the positive and negative ions produced in a CI source are alternately pulsed from the source, with appropriate potentials, through a quadrupole analyzer to two electron multipliers, one for positive and one for negative ions. Positive and negative mass spectra are, thereby, measured "simultaneously". Under favorable circumstances, negative CI can afford sensitivity two or three orders of magnitude greater than that obtainable with positive CI (131), making it a very relevant technique for residue analysis of pesticides and dioxins.

The positive and negative methane (132) and isobutane (133) CI mass spectra of selected polycyclic and aromatic chlorinated insecticides of several types have been determined and published. The negative CI spectra with isobutane as enhancement gas were exceptionally simple, with the most abundant ion for almost all compounds studied being $(M + Cl)^-$ (133).

Negative CIMS with methylene chloride reagent gas was the basis of a multiresidue screening procedure for OCl residues in environmental substrates at 1 ng levels (134). The four principle negative ion-forming reactions with methylene chloride as reagent gas were (1) resonance capture of an electron to give $M^{\bar{\cdot}}$; (2) chloride attachment to hydrogen-bonding or carbon-bonding substrates to give $(M + Cl)^-$; (3) deprotonization or dissociative capture of an electron for relatively strong gas phase acids to give $(M - H)^-$; and (4) oxygen-chloride exchange to give $(M - Cl + O)^-$ (113A, 134).

Polychlorinated dibenzo-p-dioxins were determined in biological samples by methane negative CIMS, which was found to be as much as 1000-fold more sensitive than methane positive CI and electron impact MS. The use of oxygen with or without methane resulted in decreased sensitivity but increased selectivity for the dioxins. Detection limits ranged from 100 to 500 pg for 2,3,7,8-TCDD down to ca 1-10 pg for 1,2,3,4,6,7,8-HpCDD by selective ion monitoring (131). The highly toxic 2,3,7,8-TCDD can be distinguished from other isomers by negative chemical ionization and reaction with oxygen to form dichloroquinoxide ions (134A, 134B).

A discussion of 13 methods for ionization of organic compounds in MS has been published, including detailed consideration of chemical ionization and field ionization and pesticide spectra (135). Design considerations of EI, CI, FI, FD, and API sources have been described (136). Five ionization methods were compared for producing positive and negative ion mass spectra of typical organophosphorus pesticides. The negative ionization techniques were much more sensitive for the 16 compounds tested (137).

(4) Mass Analyzer Systems

Low resolution magnetic analyzer systems depend on bending of the ion beam in a magnetic field. The magnetic field segregates the ions into beams, each of a different m/z. To obtain the mass spectrum, the magnetic field is varied, and each m/z ion from light to heavy is successively brought to focus on the exit slit. Such analyzers are referred to as single- or direction-focusing analyzers. High resolution instruments have an analyzer region with an electrostatic sector for velocity or kinetic energy focusing plus a magnetic sector for separation of fragments according to m/z ratio.

Quadrupole analyzers are based on mass separation in a radio frequency (RF) electric field. This field is established on a set of four precision parallel, usually circular, rods, with both a DC voltage and an RF alternating voltage being applied to these rods. Ions are accelerated gently (5-30 V) into the analyzer or filter region and begin to oscillate between the rods. At a given DC and RF level, ions of a specified m/z value undergo stable oscillations and pass through the length of the analyzer tube to the detector. Ions of lower or higher mass will undergo increasingly erratic oscillations that eventually result in their striking the rods or walls. The spectrum is obtained by sweeping the applied RF voltage and DC ramp voltage and measuring the detector current as a function of time.

(5) Resolution

Resolution describes the performance of the mass analyzer in terms of its ability to separate ions of different masses from one another. Resolution is expressed in numerical form by the equation $M/\Delta M$ where M and $M + \Delta M$ are mass numbers of two neighboring peaks of equal intensity in the mass spectrum. The criterion for resolution is a relative height of the valley between peaks of 10%, with each peak contributing 5% to the valley. For example, an instrument would have a resolution of 100 if two peaks with a mass difference of 1 part in 100 (e.g., m/e 100 and 101) were resolved to the 10% level. Low resolution mass spectrometers typically show maximum resolution values between 300 and 1000, while high resolution instruments are capable of attaining resolutions well in excess of 10^4. The advantage of a high resolution spectrometer is the capability of resolving ions with very little differences in mass and obtaining the masses of these ions accurately to 0.001 mass units or better. Exact masses are determined using a computer coupled to the mass spectrometer or by peak matching known marker peaks and unknown peaks on an oscilloscope (138). Once the exact mass of a key ion (often the molecular ion) is known, the elemental composition or formula of the molecular or fragment ion is obtained, again by using a computer or by consulting tabulations of the masses of different combinations of atoms. Elements indicated to be present by the mass spectral pattern or prior information about the unknown sample are often needed to correctly evaluate the data.

Resolutions of the order of 1000 are attainable with low resolution magnetic and quadrupole analyzer designs, although single-focusing magnetic analyzers can attain higher resolutions with an extreme decrease in sensitivity due to the narrow slits that must be used. Resolution in excess of 8000 is considered high, since this is the amount usually necessary to resolve most mass doublets. The extra focusing added in a high resolution mass spectrometer reduces the overall number of ions traversing the instrument, thus reducing the overall sensitivity. To overcome such a reduction, the mass range is usually scanned at a slow rate. To minimize the effects from slow scanning and decreased sensitivity, only as much resolution as is necessary to perform the required analysis should be used, since the accuracy of an exact mass measurement is independent of resolution as long as any mass doublets are separated.

References (108, 110, 139-141) review methods and applications of MS and combined GC/MS to pesticide residue analysis, and references (111, 112) give a more general survey of GC/MS instrumentation, principles, and techniques.

b. Examples of GC/MS Confirmation

Figure 10-J shows the electron capture gas chromatogram obtained by injection of an aliquot of the 6% ethyl ether Florisil column eluate from cleanup of a human adipose tissue extract (142). Figure 10-K shows the total ion current chromatogram of the same eluate from GC-MS. Although the curves are drawn to different scales and are not directly comparable, it is evident that many more compounds are identifiable in the latter because of the general response of the mass spectrometer. In general, chromatograms traced by the total ion monitor are similar, but not necessarily identical, in response and sensitivity to those traced by a flame ionization detector. Differences exist in sensitivities to some compounds, and broadening occurs in some peaks in the interface to the mass spectrometer. Figure 10-L shows the mass spectrum of standard *p,p'*-DDE, the major GC peak evident in both chromatograms in Figures 10-J and 10-K.

The identification of pesticides from their mass spectra is often complicated by the obscuring of low mass ions by impurity fragments, especially in biological extracts. For this reason, extra cleanup of extracts may be needed for GC-MS as compared to GC alone. For example, alkaline hydrolysis has been used for the 15% ethyl ether Florisil column eluate, while additional column adsorption cleanup (e.g., alumina plus Florisil columns) or use of silica gel rather than Florisil initially has been successful for the 6% ethyl ether eluate. Gel permeation chromatography has also been successfully applied to the 6 and 15% fractions (143).

Figure 10-J. Electron capture chromatogram of human adipose tissue extract, 6% ether Florisil column eluate

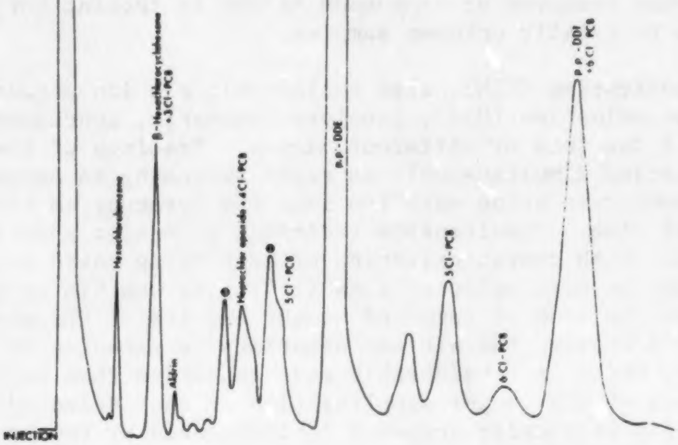
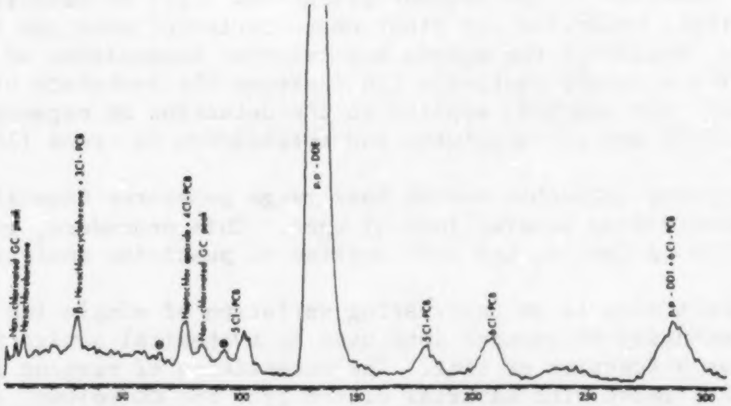


Figure 10-K. Total ion current profile of the same human adipose tissue extract



c. The Mass Spectrometer as a GC Detector

There are a number of ways to use the mass spectrometer as a sensitive and selective GC detector. These procedures require that the analyst know what compound or compounds he/she is looking for and are not applicable to totally unknown samples.

Selected ion monitoring (SIM), also called multiple ion detection (MID) or multiple ion selection (MIS), involves automatic, continuous monitoring of a few ions of different masses. Tracings of the selected masses are recorded simultaneously as rapid switching is accomplished in the spectrometer to bring each ion into the detector in turn for a short period of time. Simultaneous recording of one or several compounds can be achieved, with characterization of each being based on the formation of one or more selected ions (144). To use SIM effectively, one should know the kind of compound sought and its MS characteristics. Sensitivity of detection for SIM can sometimes be extended to the subpicogram range, which is considerably more sensitive than conventional scanning because of the longer sampling time at each selected mass. Sensitivity for a particular compound is influenced by the extent of fragmentation and the fraction of the total ion current carried by the selected ions. Identification and quantitation of compounds can be improved by exact mass measurement (e.g., to 0.001 amu) of the specified ion, but only at the expense of sensitivity (144A). Figure 10-M shows the m/z 405, 407, 409, and 411 ions of trans-nonachlor and isomers monitored simultaneously in a human adipose tissue. Total ion current profiles (TICP) cannot be generated by the SIM technique because data from only certain masses are collected.

Compounds not resolved by gas chromatography can still be detected with certainty if their molecular (or other characteristic) ions can be resolved by SIM. Recording the masses and relative intensities of several ions formed from a single pesticide can increase the certainty of compound identification. SIM has been applied to the detection of organophosphorus insecticides (145) and to carbofuran and metabolites in crops (146).

Repetitive scanning through a narrow mass range generates quantifiable spectral envelopes from several ions at once. This procedure, generally sensitive at low ng levels, has been applied to pesticide analysis (147).

Reagent ion monitoring is an interesting variation of single ion monitoring, wherein the intensity of reagent ions used in a chemical ionization source is monitored as a function of time. The intensities of reagent ions decrease when they react with material eluted from the GC column, providing a chromatogram that is distinctive from those produced by other detectors (148).

Figure 10-L. Total mass spectrum of p,p'-DDE

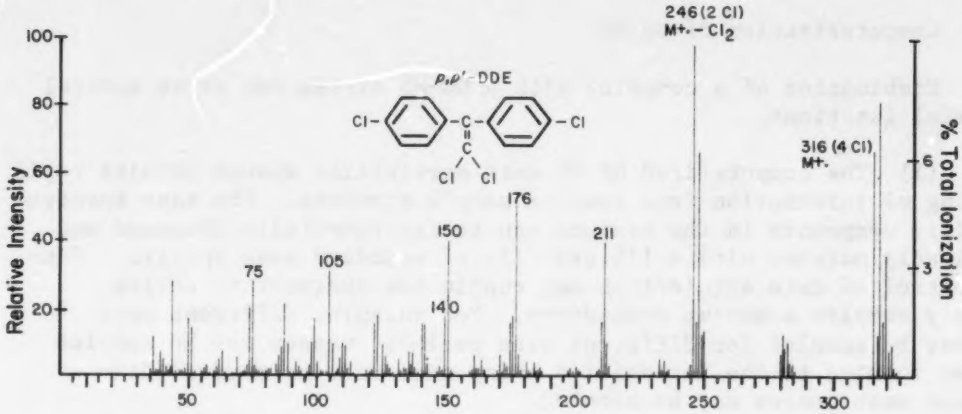
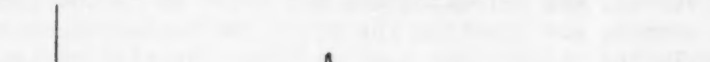


Figure 10-M. Selected ion monitoring applied to a human adipose tissue extract. The four masses shown have been monitored as the extract elutes from an OV-17/OV-210 GC column at 180°C. The largest peak is trans-nonachlor, the last eluting peak is cis-nonachlor, and the peak preceding trans-nonachlor is an isomeric nonachlor also observed to be present in technical chlordane.



d. Computerization of GC-MS

Combination of a computer with a GC-MS system can serve several very useful functions.

(1) The computerized GC-MS data acquisition system permits rapid processing of information from complex sample mixtures. The mass spectra of specific compounds in the mixture can be experimentally obtained and automatically matched with a library file of standard mass spectra. Computer control of data acquisition may enable the operator to devise relatively complex scanning procedures. For example, different mass ranges may be sampled for different time periods, masses may be sampled for times related to the intensities being measured, or several discontinuous mass ranges may be sampled.

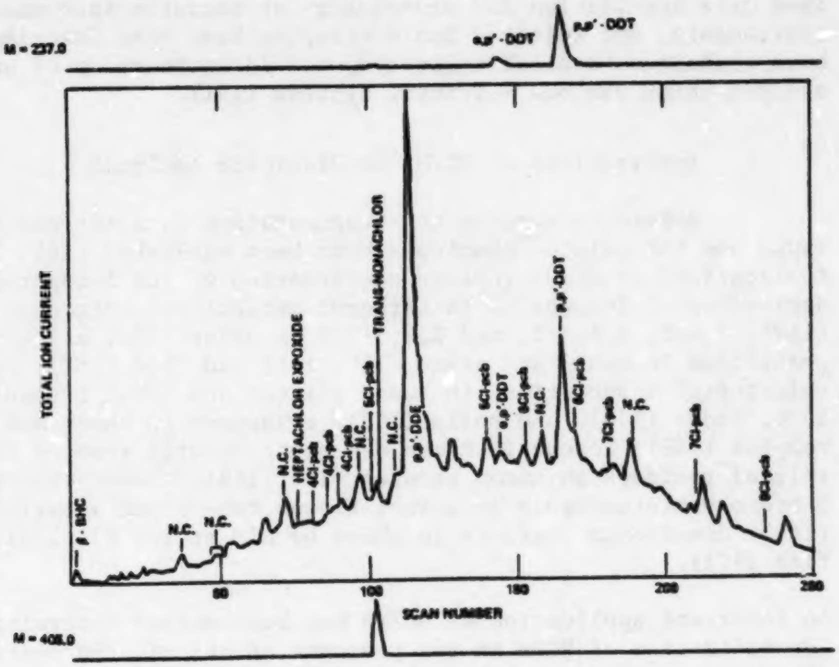
(2) Column bleed and other background can be conveniently subtracted by the computer.

(3) Continuous repetitive scans can be made during the entire chromatographic separation; for example, a spectrum can be scanned every 2-4 seconds. In a typical GC/MS run, several hundred to more than a thousand mass spectra may be acquired in this way, each one being a complete spectrum over the mass range selected.

All spectra are stored, and chromatograms may later be reconstructed by the computer by summing and plotting the total ion current detected in each scan but excluding carrier gas ions or other interfering ions. Reconstructed total ion current profile chromatograms (TICP) obtained resemble those traced in real time by a conventional total ion monitor of a magnetic deflection spectrometer. A typical reconstructed GC/MS total ion current profile of an extract of human fat is shown in Figure 10-N, with some of the components identified (142).

(4) The computer can trace the intensities of selected characteristic masses from among the great quantity of data acquired by continuous repetitive scanning. The resulting mass chromatograms or extracted ion current profiles (EICP) (149) resemble the single or selected ion profiles described earlier and permit compounds and spectra of interest to be located and the appropriate spectrum to be retrieved and plotted. EICPs can be individual selected ion current profiles (SICP) or summed sets of several masses, all extracted from scanned data. EICPs have an advantage over SIM in that large numbers of ion profiles and complete spectra can be examined rapidly after only one chromatographic separation, but this computerized acquisition of repetitively scanned spectra is of considerably lower sensitivity (as much as 10^4) than SIM because of the longer integration time characteristic of the latter method (149). Reference (140) illustrates computer-generated selected ion current profiles.

Figure 10-N. Computer reconstructed total ion chromatogram and mass chromatograms of M = 237 (o,p'-DDT and p,p'-DDT) and M = 405 (trans-nonachlor) from a composite human adipose tissue extract. Column: 45.7 m SCOT column coated with SE-30, programmed from 170-240°C at 2°C/minute (N.C. = not chlorinated).



The limited mass range chromatogram (148) is a variation of mass chromatography that has proved especially valuable in the determination of polychlorinated hydrocarbons. In this technique, the computer sums ion intensities (collected from repetitive scanning) through a limited mass range as a function of scan number or time. (The procedure has also been termed selected ion summation analysis or SIS.) For example, the molecular ion cluster of mirex, due to the contributions of ^{37}Cl from each of the 12 chlorine atoms, is spread over a range of more than 27 μ. Instead of treating a single ion (e.g., $C_{10}{}^{35}Cl_{12}{}^{+\cdot}$, nominal m/z 540), the entire cluster can be summed to provide increased sensitivity with some sacrifice in specificity. The method has been used to identify dieldrin and HCB residues in lake trout (150).

(5) Quantitation of peak areas in the selected ion profiles and ratios of these peaks can be provided.

Computer coupled GC/MS equipment is extremely expensive, and highly qualified personnel are needed for operation, maintenance, and interpretation of data. A significant amount of "down-time" is to be anticipated because of the complex nature of the instrumentation. Computerized data acquisition and processing for magnetic instruments, quadrupole instruments, and selected ion monitoring have been described (110), as have techniques available for computer identification of unknown mass spectra using various retrieval systems (151).

e. Applications of GC/MS to Pesticide Analysis

Reference spectra and fragmentation data for pesticides of several types and for related chemicals have been published (105, 108, 152-156). Applications of GC/MS include confirmation of the 1-naphthyl chloroacetate derivative of 1-naphthol (a carbaryl metabolite) extracted from urine (157); 2,4-D, 2,4,5-T, and 2,4,5-TCP in urine (158, 159); organophosphorus pesticides in blood and urine (160, 161) and food (162); multiple chlorinated insecticides in human adipose and liver tissue (142, 143, 163), foods (164), and soils (165); toxaphene in human and biological samples (166); Kepone in human and environmental samples (167); chlordane-related residues in human samples (142, 168); thiabendazole and 5-hydroxythiabendazole in animal tissue (on-column methylation plus SIM) (169); dimethoate residues in wheat by SIM at m/z 87 (170); and mirex in fish (171).

An important application of GC/MS has been mutual determination and identification of PCBs in the presence of chlorinated pesticides (172). Insecticides mixed with PCBs have been identified at levels below 10 ng without complete separation on a GC column by peak monitoring MS as described earlier (173). GC/MS has been successfully applied to the detailed analysis of complex pesticide mixtures, such as technical chlordane (168). Pesticides and PCBs have also been identified by GC/MS using chlorine isotope ratios to reconstruct chromatograms that are characteristic for the number of chlorine atoms found in repetitive-scan spectra (174).

Special MS and GC/MS techniques that have been applied to the analysis of simple and complex pesticides in a variety of sample substrates include selected ion monitoring (175, 176), field ionization (177), and field desorption MS (178). Methods have also been developed for the determination of carbamates and ureas by combined liquid chromatography/mass spectrometry (117). References (108, 110-112, 179) contain reviews of applications to residue analysis. Symbolism and nomenclature of mass spectrometry have been reviewed (107).

f. Mass Spectrometry/Mass Spectrometry (MS/MS)

Mass spectrometrists have, within the last few years, investigated the possible elimination of any preseparation method, such as GC or LC, for the analysis of complex mixtures. Instead, the mass spectrometer itself is used as the separation device, followed by a second mass spectrometric analysis of the sample. This technique is called mass spectrometry/mass spectrometry. Techniques are available to perform this method using quadrupole or magnetic sector instruments with positive or negative ions. Operation commonly involves the separation of the ions of a particular m/z value, characteristic of a given compound present in a complex mixture, by a first mass spectrometer. This ion current then encounters collisions with gas molecules, which impart considerable energy to them through the process of collisional activation. The resulting energetic ions may then decompose into characteristic fragments, which are then analyzed in a second or third mass analyzer region, as the case may be. This method holds promise as a rapid method of mixture analysis. Hunt et al. (179A) have used MS/MS to analyze nitrophenols in sewage sludge. However, recent studies show that artifacts can be created in the analysis (111). Other references involving MS/MS include (179B, 179C).

10M QUALITY ASSURANCE OF GC-LOW RESOLUTION MASS SPECTROMETRY

This section reviews procedures to be followed for quality assurance of data derived from the mass spectrometer in the identification, confirmation, and quantitative determination of chlorinated insecticides, PCBs, hexachlorophene, and PBBs in human tissues and fluids. These methods were developed at the Health Effects Research Laboratory, U.S. EPA Research Triangle Park, NC (180) for use in the EPA National Human Monitoring Program for adipose tissue and serum samples. The procedures assure interpretable mass spectra of the highest experimentally obtainable quality for compound identification as well as quantitative accuracy when monitoring ion intensities (as by selected ion monitoring). The specific pesticides of current interest are the following:

o,p'-DDT
p,p'-DDT
o,p'-DDE
p,p'-DDE
o,p'-DDD
p,p'-DDD
α-BHC
β-BHC
Lindane (γ-BHC)
δ-BHC

Aldrin
Dieldrin
Heptachlor
Heptachlor epoxide
Endrin
Mirex
Oxychlordane
trans-Nonachlor
Polychlorinated biphenyls
Hexachlorobenzene
Polybrominated biphenyls
Polychlorinated terphenyls

a. Introduction to Quality Assurance Procedures

Correct identification of organic pollutants from gas chromatography-mass spectrometry (GC/MS) data requires valid mass spectra of the compounds detected. This is independent of the actual method of interpretation of the spectra, i.e., an empirical search for a match within a collection of authentic spectra or an analysis from the principles of organic ion fragmentation. A properly operating and well tuned GC/MS instrument is required to obtain valid mass spectra.

The purpose of the following procedure is to permit a check of the performance of the total operating computerized GC/MS system. Thus, with a minimum expenditure of time, an operator can be reasonably sure that the GC column, the enrichment device, the ion source, the ion separating device, the ion detection device, the signal amplifying circuits, the analog to digital converter, the data reduction system, and the data output system are all functioning properly.

An unsuccessful test requires the examination of the individual subsystems and correction of the faulty component(s). Environmental data acquired after a successful system check are, in a real sense, validated and of far more value than unvalidated data. Environmental data acquired after an unsuccessful test may be worthless and may cause erroneous identifications. It is recommended that the tests be applied often on a working system, especially when there is a suspicion of a malfunction.

The procedure is written for a low resolution mass spectrometer such as the Finnigan 3200 or the Hewlett Packard 5930A quadrupole-type mass spectrometer, equipped with an automated data system such as the Finnigan 6000 or Hewlett Packard 5933A system. However, the test is clearly and readily adapted to any GC/MS system by suitable modification of the detailed procedure.

There is a special need to closely monitor the performance of the quadrupole mass spectrometer. Unlike the magnetic deflection spectrometer, the active ion separating element of a quadrupole spectrometer (the rods) is directly contaminated during operation, and after prolonged operation is subject to severely degraded performance. Since degraded performance usually affects the high mass region first, the test includes high mass end criteria. High quality, high mass data are important since many environmentally significant compounds have molecular and fragment ions in the 300-500 µ range.

A quadrupole mass spectrometer, which has been tuned to give a reference compound spectrum that meets the criteria of this test, will, in general, generate mass spectra of organic compounds that are very similar, if not identical, to spectra generated by other types of mass spectrometers. Thus, quadrupole mass spectra will be directly comparable to spectra of authentic samples in collections that have developed over the years, mainly from magnetic sector mass spectrometers.

Assurance of mass spectral data is obtained through a set of two levels of functionality tests. The first test requires establishment of production, dispersion, and detection of ions from a reference compound, perfluorotri-n-butylamine (PFTBA). Relative peak heights are adjusted to conform to the known electron impact spectrum, with a slight biasing toward increased transmission of ions higher than m/z = 200, which are not commonly interfered with by tissue component fragments.

The second test of the GC-MS combination requires injection of a known low-level standard sample while the operation is under computer control. This is followed by periodic verification of the quality of spectra compared to spectra of known ideal quality. Chemical compounds used may be bis(perfluorophenyl)phenylphosphine(or decafluorotriphenyl phosphine, DFTPP). Another set of compounds commonly used are aldrin and heptachlor epoxide. Heptachlor epoxide is useful as a representative member of the important chlordane series of pesticides and, more generally, because the M-Cl ion, six-chlorine isotope cluster beginning at 351 m/z allows a test of sensitivity and resolution at a very useful mass, not provided for by PFTBA or many other mass calibration compounds, but quite relevant to pesticide work. The appearance of the 351 m/z cluster may be examined at 100, 10, and 1 ng levels, as instrument sensitivity requires, with respect to appearance of the six-chlorine cluster versus statistical appearance. Resolution of ^{13}C isotope peaks and relative abundance versus the 81 m/e peak may also be determined. Aldrin, injected as a GC retention time test, also has its mass spectrum routinely compared against the literature spectrum with respect to correctness of chloro cluster statistics, sensitivity, and relative appearance of high and low mass fragment ions. The retention time of heptachlor epoxide relative to aldrin (1.59 ± 0.02) on a 1.5% OV-17/1.95% OV-210 column at 185°C may also be determined, along with GC column resolution. This test has the advantage of providing a full functionality evaluation of the GC/MS system, including sensitivity, data system acquisition, and recall of spectra.

b. Quality Assurance Procedures

(1) Using PFTBA (3M trade designation: FC-43) standard:

(a) Check on oscilloscope and/or light beam oscillograph that 69, 131, 219, 264, 414, 502, and 614 m/z ions are present and in reasonable relative abundance according to the following tabulation:

General Desired Appearance of the Mass Spectrum of PFTBA

Mass (m/z)	Relative Abundance %
69	100.0
100	24.3
114	9.5
119	20.3
131	70.3
219	68.0
264	16.2
414	5.4
426	2.7
502	2.7
614	0.3

^{13}C Isotope Abundance Checks, Percentage Ratio of Ion Signal Abundances

(70)/(69) = 1.1%

(220)/(219) = 4.4%

(503)/(502) = 10.3%

(b) Tune mass spectrometer as required, with respect to resolution, optimum peak shape, sensitivity, and minimum mass falloff (refer to appropriate instrument manual for instructions).

(c) Calibrate data system and verify the calibration by examining a PFTBA spectrum acquired under data system control (refer to appropriate data system manual for programs).

(2) Run aldrin and/or heptachlor epoxide and examine the reconstructed total ion chromatogram and mass spectra.

(3) Perform DFTPP test (optional).

(4) Go on to sample runs.

c. Preparation of Aldrin and/or Heptachlor Epoxide Standards

Primary standards of aldrin and heptachlor epoxide can be obtained from the Pesticide Repository, Health Effects Research Laboratory, EPA, Research Triangle Park, NC.

Carefully weigh out 20 mg of the pesticide and dissolve in 100 ml of n-hexane (pesticide quality, or equivalent) in a volumetric flask. Keep this stock solution under refrigeration. Replace every 6 months.

Prepare a working standard of 20 ng/µl concentration by diluting 1 ml of the stock solution to 10 ml in a volumetric flask. These working solutions should be replaced at least monthly.

d. Preparation of Decafluorotriphenylphosphine (DFTPP) Standards

Prepare a stock solution of DFTPP at 1 mg/ml concentration in acetone (pesticide quality, or equivalent). This stock solution has been shown to be 97+ percent stable after 6 months, and indications are that it will remain useable for several years. Dilute an aliquot of the stock solution to 10 µg/ml (10 nl/µl) concentration in acetone. The very small quantity of material present in very dilute solutions is subject to depreciation due to adsorption on the walls of the glass container, reaction with trace impurities in acetone, etc. Therefore, this solution may be useable only in the short term, perhaps 1-3 weeks.

e. Quality Assurance Test

(1) Adjust the GC column flow to normal operational level (e.g., 30 to 45 ml/min) and set the desired oven temperature (e.g., 185°C). The parameters should be adjusted to provide at least four spectral scans during the elution of the aldrin, heptachlor epoxide, or DFTPP standard.

(2) Set mass spectrometer at normal or high sensitivity as desired.

(3) Calibrate the instrument.

(4) Inject 40 ng of aldrin and/or heptachlor epoxide (or 20 ng of DFTPP) on the GC column and note the time (or start stopwatch).

(5) After the solvent passes through the analyzer and the vacuum has recovered, turn on the ionizer and start scanning.

(6) Note the exact retention time of the standard as it elutes from the column. This retention time can be used as a daily check of the condition of the GC column and separator by comparing the values. The retention times should not vary significantly from day to day under identical operating conditions.

(7) Terminate the run, turn off the ion source and electron multiplier, and reconstruct the gas chromatogram.

(8) Select a spectrum number on the front side of the GC peak as near the apex as possible and select a background spectrum number immediately preceding the peak.

(9) Plot or display the mass spectrum and compare against a reference spectrum. The spectrum obtained on the test system should contain ion abundances within limits given for the key ions in the following tables. Sensitivity is considered adequate if 40 ng or less of either aldrin or heptachlor epoxide and 20 ng or less of DFTPP provide good mass spectra.

Reference Aldrin Mass Spectrum
(5-chlorine cluster check)

m/z	Abund. (%)
261	61.5
262	4.7
263	100.0
264	7.8
265	65.0
267	21.1
269	3.4
271	0.2

$$\frac{(\text{intensity of fragment})}{(\text{intensity of base peak})} = \frac{(261)}{(66)} = 45\%$$

Reference Heptachlor Epoxide Mass Spectrum
(6-chlorine cluster check)

m/z	Abund. (%)
351	51.2
352	5.6
353	100.0
354	11.2
355	81.2
357	35.2
359	8.5
361	1.1
363	0.06

$$\frac{(\text{intensity of fragment})}{(\text{intensity of base peak})} = \frac{(351)}{(81)} = 47\%$$

Reference Mass Spectrum of DFTPP

Mass	Ion Abundance Criteria
51	30-60% of mass 198
68	Less than 2% of mass 69
70	Less than 2% of mass 69 (1.1% theoretical)
127	40-60% of mass 198
197	Less than 1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198 (6.6% theoretical)
275	10-30% of mass 198
365	1% of mass 198
441	Less than mass 443
442 ($M^{+\cdot}$)	40-60% of mass 198 -- this ion is very sensitive to spectrum number chosen and condition of equipment. If greater than 60%, equipment is OK if all other criteria are met.
443 (M+1)	17-23% of mass 442 (19.8% theoretical)
444 (M+2)	1.86% (theoretical)

f. Protocol for Analysis of Samples

(1) Sample Collection

Samples of human adipose tissue are obtained through cooperating medical pathologists and medical examiners at hospitals in cities selected according to a proportionate, stratified-random design. The conterminous 48 states were divided into 9 census divisions, according to the 1970 census of the United States. A city within each census division was selected from those already participating in the National Human Monitoring Program as the collection site for special projects.

Blood sera samples are collected throughout the U.S. by means of a cooperative arrangement between EPA and the U.S. Public Health Service. The PHS program, called the Health and Nutritional Examination Survey II (HANES II), provides blood specimens from a probability sample of persons 12 to 74 years old, along with various medical and nutritional parameters and some information regarding pesticide use by the individuals sampled. The blood is drawn into evacuated ampoules, allowed to clot, and centrifuged, and the serum is decanted into a clean vial.

(2) Cleanup

Tissues are normally extracted and cleaned up according to a modified Mills-Onley-Gaither procedure (Subsection 9A) by laboratories under contract to the National Human Monitoring Program. Concentrated extracts, corresponding to the 6% and 15% ethyl ether in petroleum ether fraction from the Florisil cleanup column, are then sent to the ACB/HERL-RTP for GC/MS analysis. Composite samples, comprising 100-500 individual samples, require additional cleanup before GC/MS analysis. The usual method of choice is gel permeation chromatography (GPC) as described in Section 9I. Blood sera samples (Section 9D) may or may not need GPC cleanup.

(3) Analysis

After cleanup, samples are concentrated by removal of solvent at room temperature under a gentle stream of nitrogen. The final volume is usually 100 µl, but it may be smaller if levels of compounds sought are particularly low. Quantitative analysis is performed in the electron impact (EI) mode. Aliquots of 5 to 50 µl are co-injected with aldrin (e.g., 250 ng in hexane) as an internal standard into the GC/MS system. A total ion current profile is generated, and retention times relative to aldrin are determined for each component of interest. Mass spectral data are recalled from the computer for each component of interest and analyzed against reference mass spectra obtained from various literature references (e.g., 142, 143) or from a reference library such as the NIH-EPA Chemical Information System (181), or, most preferably, generated from authentic laboratory standards. Relative retention times are also compared to those of the reference material for further confirmation.

After identification, quantitative analyses are usually performed by selected ion monitoring (SIM). An authentic reference sample is used for direct comparison. Identification may be further confirmed by chemical ionization GC/MS, where available.

g. GC/MS Systems

Manufacturer's operating manuals should be consulted for descriptions and detailed operating instructions for specific GC/MS systems. The previous edition of this Manual contained information on two GC-MS systems: the Hewlett Packard 5930A quadrupole MS, 5700A gas chromatograph, and 5933A data system; and the Finnigan 3200 quadrupole MS and 9500 gas chromatograph.

Another EPA Manual (182) contains specific information on the Finnigan 1015 and 3000 quadrupole GC/MS systems coupled with a PDP-8 data system. This Manual includes 10 chapters covering the following material: (1) introduction to broad spectrum organic analysis, routine monitoring of large numbers of target compounds, and real time selected ion monitoring; (2) detailed start-up and calibration procedures; (3) preparation methods for water samples; (4) information on OUTPUT programs for data analysis; (5) compound identification using PDP-8 software; (6) specialized techniques such as single ion monitoring, open tubular columns, chemical ionization, accurate mass measurement, standard additions, and sample spikes; (7) miscellaneous auxiliary software programs and housekeeping routines; (8) preventive maintenance; (9) trouble shooting; and (10) selected bibliography up to 1978, mostly to information from EPA laboratories.

10N BIOLOGICAL METHODS

Bioassay techniques, which include insecticidal activity, enzymatic, and immunological methods, have been described as providing an independent criterion of identity when combined with GC, chemical reactions, etc. (2). These methods, which depend on the measurement of a physiological response of a test organism induced by exposure to the pesticide, have advantages of simplicity and sensitivity but are relatively non-specific so that their utility for confirmation is rather poor. The insect bioassay technique has been reviewed (183).

Specificity of enzyme inhibition is greatly enhanced by combination with TLC for detection and confirmation of organophosphate and certain carbamate pesticides. The R_F value plus biological response provide important identity information at levels typically in the range of 500 pg to 10 ng for these compounds.

100 POLAROGRAPHY (VOLTAMMETRY)

Voltammetry is the generic name for a group of electroanalytical methods in which current-*vs*-voltage curves are recorded when a gradually changing voltage is applied to a cell containing the solution to be analyzed, a stable reference electrode, and a small-area working or indicator electrode. In the special case where the indicator electrode is a dropping mercury electrode, the technique is called polarography. In addition to classical DC polarography, in which the current is measured for each drop as voltage is increased linearly with time, modern variations include DC current sampled polarography, pulse polarography, differential pulse polarography, linear sweep (rapid scan) polarography, and AC polarography. These newer methods differ in the type of voltage signal applied and/or the manner in which the current is measured, and they are generally more sensitive and/or selective than traditional DC polarography.

The use of polarography as a confirmatory test is described in Section 12,F of the EPA PAM and Sections 640 and 641 of the FDA PAM. Procedures and applications of polarography for both identification and determination of pesticide residues have been reviewed (184-186).

Polarographic identification of a pesticide residue is based on the determination of the peak potential of the unknown in a cleaned-up extract, and comparison with the potential of about the same amount of a reference standard under identical conditions. As a check, addition of the standard compound to the unknown should result in an increase in the wave height but not appearance of another wave. Mixtures can be identified if the peak potentials of the components are sufficiently separated. Trapped GC fractions may be subjected to polarography to confirm identifications based on retention times. Instrumentation for such modern voltammetric techniques as fast sweep oscillography provides sensitivity comparable to colorimetry. Pesticides not containing an oxidizable or reducible functional group can be made amenable to polarography by formation of a suitable derivative (e.g., nitro, halogen, carbonyl, etc).

Most polarographic studies have been applied to phosphorus-containing insecticides such as parathion, diazinon, malathion, and carbophenothion (187). A collaborative study confirmed the usefulness of single sweep oscillographic polarography for identifying such residues in non-fatty foods (188). Nitrophenol metabolites of OP pesticides were determined in urine by polarography (189). Thirty-eight herbicides have been studied by single sweep derivative polarography (190), methylcarbamate insecticides by AC polarography and cyclic voltammetry (191), and urea herbicides by anodic polarography (192). Published voltammetric reduction potentials for about 100 organochlorine insecticides, PCBs, and naphthalenes (3 electrode potentiostat, DMSO solvent) are a useful aid in identification of residues (193). Parathion and related insecticides and metabolites were polarographically determined in blood without extraction (194). The voltammetry of 1,3,5-triazines (195), propachlor herbicide (in soil) (196), dithiocarbamates (197, 198), dinitroaniline herbicides (199), thiourea-containing pesticides (200), trifluralin (in soils) (201), azomethine-containing pesticides (e.g., Cytrolane, Cyolane, chlordimeform) (202), PCP (203), and phosmet (in apples) (204) has been reported. Paraquat can be directly determined in urine and serum by differential pulse polarography at ca 0.04 μg/ml levels (205).

10P MISCELLANEOUS CONFIRMATORY METHODS

a. Carbon Skeleton Chromatography

Carbon skeleton chromatography (CSC) is useful in characterizing insecticide residues in amounts down to 5-100 ng. Apparatus for CSC consists of a precolumn containing a hot (ca 300°C) catalyst attached to a gas chromatograph equipped with a flame ionization detector (available from National Instruments Laboratory, Rockville, MD). The compound to be identified is injected directly on the catalyst bed (e.g., 1% Pd on 60-80 mesh Gas-Chrom P) and is swept over the bed by hydrogen carrier gas. Nitrogen is introduced through the normal instrument inlet so that the detector yields optimum response. While in the precolumn, all functional groups are stripped from the compound, and any multiple bonds are saturated. The resulting hydrocarbons are carried into the chromatographic column where they are separated and identified by their retention characteristics relative to standards. This identification method, which is in effect a derivatization procedure, has been applied to heptachlor, heptachlor epoxide, chlordane, aldrin, endrin, DDT and its analogs, and carbaryl. Sufficient residue must be available for the method to be of value. Techniques, applications to many pesticide classes, and characterization of products of CSC (as well as some other precolumn reaction confirmatory methods) have been reported by Beroza and co-workers (206-209) and Asai *et al.* (210, 211). Identification of 5-10 ng amounts of polychlorinated biphenyls, terphenyls, naphthalenes, dioxins, and dibenzofurans in biological samples has been demonstrated (212), and mixtures of polychlorinated naphthalenes, PCBs, PCTs, and OCl pesticides have been analyzed (213).

b. Fragmentation Procedures

GC fragmentation procedures are similar to CSC except that the reaction in the precolumn decomposes the pesticides, yielding characteristic fragment peak patterns or fingerprint chromatograms helpful in making identifications. A palladium catalyst at 300°C (210) and reagents such as Na_2CO_3, CuO, $CdCl_2$, $AlCl_3$, and $K_2Cr_2O_7$ at 240°C (214) have been applied to chlorinated and OP insecticides with EC detection of the reaction products.

Gas chromatograms of 33 organochlorine pesticides after ultraviolet irradiation have been published. These characteristic photodecomposition patterns are also useful for conclusive residue confirmation (215).

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Section 11

TRAINING OF PESTICIDE ANALYTICAL CHEMISTS

This chapter is by far the shortest in the manual, and the reader may question the logic of devoting a special section to this subject. During the years of operating the interlaboratory quality control program described in Section 2, the editors observed overwhelming evidence that participating laboratories with chemists who had formal, specialized training demonstrated far superior analytical performance than did those laboratories which lacked this advantage. We, therefore, regard training as a highly important subject and deserving of special treatment.

Although many good programs are available in undergraduate and graduate schools for the training of analytical chemists, few, if any, specifically train pesticide analysts. An undergraduate or graduate student on a research project with a professor interested in development of residue analytical methods does receive valuable training and experience, but such professors are few and far between in American education institutions. A number of companies in the private sector offer short courses particularly designed for training users of company-produced equipment. A certain few universities and private educational organizations run short courses touching upon a few of the highlights of pesticide residue analysis. Some governmental agencies operate similar short training courses.

The residue chemist must not only be familiar with the technique of trace analysis in general and of residue analysis in particular, but he must be able to perform routine service and adjustments and preventative maintenance, such as module replacements and replumbing, on his instruments. In order to achieve these abilities, a generally trained analytical chemist should be given on-the-job training by an experienced residue chemist when he is hired, if at all possible. Since this is often not possible, especially in smaller laboratories, this Manual is designed to substitute, in small part, for such training and to help the analyst recognize certain pitfalls and to better perform analyses of biological and environmental media. There is, however, no really satisfactory substitute for intensive, practical bench training of the type formerly provided by the EPA Perrine Primate Laboratory Training Program, Perrine, Florida. During the years of conducting the interlaboratory quality control program described in Section 2, it was very apparent that those laboratories which took most advantage of the Perrine training facility recorded far better analytical performances on round robin samples than laboratories not participating in the training program. As a specific illustration of this, the reader is referred to Table 11-1 (copied from Table 2-15 in Section 2) which lists the relative performance ranking of 34 laboratories in one interlaboratory check sample exercise.

The eight laboratories with top performance had previously sent personnel to the Perrine training program. Of the 17 laboratories in the top half of the table, 10 of these laboratories had Perrine-trained chemists. Of the 17 laboratories in the lower quality half of the table, only one laboratory near the top of the lower half had sent personnel for training. All laboratories which had Perrine-trained personnel are check-marked next to their identifying code numbers.

The editors feel that the data shown by this table provide most conclusive evidence of the value of a proper training program in the potential quality output of a pesticide analytical chemist. Unfortunately, however, the agency saw fit to discontinue the Perrine training program, the only one of its kind in existence, and, as stated in Section 2J, some recent results on interlaboratory quality assurance fat check samples (see Table 2-23) indicate the need for a training program.

It is hoped that some educational institutions or governmental agency will recognize the need and set up programs to provide such training, and that laboratory supervisors will take advantage of these in urging their residue chemists to obtain and refresh, on a continuing basis, their training and knowledge in analytical and instrumental areas. Rapid developments in instrumentation and new techniques, and the need to analyze at lower and lower levels for an ever increasing number of pesticides and metabolites, dictate a constant need for training and retraining in a field as highly complex as that of residue analytical chemistry. Furthermore, recent disclosures of pollution of the nation's air and water by a wide variety of organic compounds, including pesticides, point up the need for scientists with a sound background of analytical expertise.

TABLE 11-1

RELATIVE PERFORMANCE RANKINGS

CHECK SAMPLE NO. 26, MIXTURE IN SOLVENT

Lab. Code Number	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
✓161.	0	0	0	198
✓137.	0	0	0	198
✓135.	0	0	0	197
✓162.	0	0	0	197
✓87.	0	0	0	197
✓113A.	0	0	0	197
✓113.	0	0	0	196
✓85.	0	0	0	196
48.	0	0	0	195
130.	0	0	0	195
✓66.	0	0	0	195
73.	0	0	0	194
✓72.	0	0	0	194
84.	0	0	0	192
89.	0	0	0	192
88.	0	0	1	189
83.	0	0	0	189
96.	0	0	2	187
97.	0	1	0	181
164.	0	1	4	169
✓68.	1	0	1	168
92.	1	0	0	168
93.	0	1	2	164
90.	1	0	2	159
53.	1	0	1	158
163.	1	1	0	157
95.	2	0	0	146
160.	2	1	0	133
45.	0	0	6	128
71.	3	0	3	127
52.	2	1	2	123
47.	3	0	1	115
69.	4	0	2	84
54.	4	4	4	25

1/ Values outside confidence limits

2/ Total possible score, 200 points

Section 12

ABBREVIATIONS*

α	Selectivity
AFID	alkali flame ionization detector
AFS	amperes full scale
API	atmospheric pressure ionization
AR	analytical reagent

BGS	detector background signal
BHC	hexachlorocyclohexane
BHT	butylated hydroxytoluene

°C	degrees centigrade
CCD	Coulson conductivity detector
CDEC	sulfallate
CI	chemical ionization
cm	centimeter
conc.	concentrated
DCNA	dichloran
DDA	bis(*p*-chlorophenyl)acetic acid
CSC	carbon skeleton chromatography
CV	coefficient of variation

2,4-D	2,4-dichlorophenoxyacetic acid
DC or dc	direct current
DCB	decachlorobiphenyl
DDD	see TDE
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DDMU	*p*,*p*'-DDD, olefin
DEF	S,S,S-tributyl phosphorotrithioate
DEGS	diethylene glycol succinate
DEPP	$(C_2H_5O)_2$-PO-O-C_6H_5
DEPTP	$(C_2H_5O)_2$-PS-O-C_6H_5
DFTPP	decafluorotriphenyl phosphine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNBP	dinoseb
DNFB	2,4-dinitrofluorobenzene
DNOC	4,6-dinitro-*o*-cresol

EC	electron capture
EI	electron impact
EICP	extracted ion current profile
EPA	Environmental Protection Agency
EPN	O-ethyl O-*p*-nitrophenyl phenylphosphonothioate
ETD	Environmental Toxicology Division
ETU	ethylenethiourea
eV	electron volt

FD	field desorption
FDA	Food and Drug Administration
FI	field ionization
FID	flame ionization detector
FPD	flame photometric detector
fsd	full scale deflection
FT	Fourier transform

g	gram
GC/MS	gas chromatography coupled with mass spectrometry
GC	gas chromatography
GPC	gel permeation chromatography

HCB	hexachlorobenzene
HECD	Hall electrolytic conductivity detector
HP	high performance
HPLC	high performance liquid chromatography
HPTLC	high performance thin layer chromatography
Hz	hertz

id	inside diameter
IR	infrared
I_{sat}	maximum current from a saturated detector

k'	capacity factor
K-D	Kuderna-Danish
kg	kilogram

l or L	liter
LC	liquid chromatography

M^+	molecular ion
MC	microcoulometric
MCPA	[(4-chloro-o-tolyl)oxy] acetic acid
MCPB	4-[(4-chloro-o-tolyl)oxy] butyric acid
m/z	mass to charge ratio
mg	milligram
MID	multiple ion detection
MIS	multiple ion selection
ml	milliliter
mm	millimeter
MOG	Mills, Onley, Gaither
MS	mass spectrometry
MT	Microtek

N	number of theoretical plates
ng	nanogram
Ni	nickel
nm	nanometer
NMR	nuclear magnetic resonance
N-P	nitrogen-phosphorus

OCl	organochlorine
od	outside diameter
OP	organophosphorus

PAM	pesticide analytical manual
PBB	polybrominated biphenyl
PC	paper chromatography
PCB	polychlorinated biphenyl
PCP	pentachlorophenol
PCT	polychlorinated terphenyl
PFTBA	perfluorotri-*n*-butylamine
pg	picogram
pH	measure of acidity; negative log of H^+ concentration
PID	photoionization detector
PLOT	porous layer open tubular
PM	photomultiplier
PNP	4-nitrophenol
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
psi	pounds per square inch
p-values	partition ratio of a solute between immiscible solvents

QA	quality assurance
QC	quality control

R	resolution
RF	radiofrequency
R_F	ratio of distance moved by TLC spot to distance of solvent front
RRT	relative retention time
RSD	relative standard deviation
R_x	R_F value relative to that of a standard compound

s or SD	standard deviation
SCOT	support coated open tubular
SEU	standard error unit
SICP	selective ion current profile
SIM	selected ion monitoring
SIS	selected ion summation
SPED	sulfur-phosphorus emission detector
SPRM	standard reference material

T	total error
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
TC	to contain
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TD	to deliver
TDE	DDD; 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane
THF	tetrahydrofuran
TIC	total ion current
TICP	total ion current plot
TLC	thin layer chromatography

μ	micron; also atomic mass units
μg	microgram
μl	microliter
μm	micrometer
UV	ultraviolet

V	volts
vs.	versus

WCOT	wall coated open tubular

* For abbreviations, names, and formulas of pesticides not listed, see the U.S. EPA Analytical Reference Standards Manual (EPA-600/9-78-012).

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